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Soil Amino Acid Composition Quantified by Acid Hydrolysis and Anion Chromatography–Pulsed Amperometry

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Soil organic N accounts for 95−98% of the total soil N content with amino acids (AAs) and amino sugars (ASs) identified as the major soil organic N compounds, but traditional 6 M HCl with reflux or sealed digestions for 24 h and various detection systems have accounted for only 30−40% of soil total N content as AA-N. This study compared traditional HCl extraction methodology with methanesulfonic acid (MSA) hydrolysis and nonderivatized AA and AS quantification by ion chromatography with pulsed amperometric detection for determination of the AA composition of plant litter and soils. MSA (4 M) gave AA-N recovery comparable to or better than 6 M HCl for plant AA digestions (16 h, 121 °C, 104 kPa). Use of 4 M MSA (0.5−1.5 h, 136 °C, 112 kPa) increased the total recovery of organic N as AAs, ASs, and NH_4^+ by 46% from soils (*n* = 22) compared with 6 M HCl (12 h, 110 °C, reflux) with a MSA recovery rate of 85.6% of the total N content (*n* = 22 soils). The shorter MSA soil digestions (0.5−1.5 h) suggested that the majority of soil organic N was not present as protein forms found in plant litter analysis (16 h of digestion). MSA ion chromatographic analysis for soil AA/AS composition is a robust nonderivatization method requiring little sample preparation that can distinguish between small changes in soil AA composition during one growing season due to vegetation and tillage managements.

KEYWORDS: Soil organic nitrogen; amperometric detection; soybeans; determination; analysis; corn; methanesulfonic acid; ion chromatography

INTRODUCTION

Organic nitrogen (N) is the most important pool of soil N composing 95−98% of the total N content and is the source of plant-available mineralized N as NH_4^+ and NO_3^- (*1*). Considerable research has been conducted for identification of the different soil N pools by means of wet-chemical hydrolysis (*2*). Identified in soil organic N hydrolysates are amino acids (AAs) (33−42% of total N content), amino sugars (ASs) (5−7% of total N content), and NH_3 released by hydrolysis (18−32%) with the remainder as nonhydrolyzed or unidentified N (*3*). Fifty-five years after the first published report on soil AA composition (*4*), on average, only 50% of the soil organic N has been identified by current hydrolysis methodology (*2*). Recent work reported that AAs are a major component of the unaccounted pool of soil N (*5*), confirming that soil AAs are even more important to the total N content than previously considered.

Although nearly all of the soil total N content is present as organic N compounds and the importance of the organic N pool to the formation of plant-available N has always been assumed, few studies have been presented to confirm the role of organic N, specifically the relationship of total soil AA content and composition to crop yields. In fact, Schulten and Schnitzer (*2*) found little variability in soil AA composition and thus dismissed the usefulness of soil AA concentration as an indicator of any biological property. Other authors, Beavis and Mott (*6*, *7*), reported that small statistical changes were evident in old Rothamsted experiment soils (1880s) when compared with recent samples (1980s). Recent research by Mulvaney et al. (*8*) and Khan et al. (*9*) has found that the concentration of the soil AS fraction was an indicator of soil N responsiveness by agricultural crops.

One of the major problems for analyzing soil organic N composition is the lack of standardized hydrolysis procedures (*10*). Variables in hydrolytic conditions include (i) type and concentration of acid, (ii) time and temperature of hydrolysis, and (iii) ratio of acid to soil and possible soil or hydrolysate pretreatment. Soil AAs have been identified in water, ether, alcohol, dilute $Ba(OH)_2$, and HF−HCl extractions (*10*, *11*), but a total AA-N accounting requires rigorous acid hydrolysis to break the AA bonds present in polymeric AA compounds such as peptides or proteins.

The large number of hydrolytic variables also matches the large number of potential quantification methodologies, each with their own limitations. The quantification of AAs can employ manometer methodology for ninhydrin-CO_2 work (*10*), different steam distillation or microdiffusion techniques for

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Table 1. Properties of Plant Biomass Used in This Study

material	organic C (g kg^{-1})	total N (g kg^{-1})	C/N ratio
clover	464	44.9	10.0
prairie[a]	453	3.70	122
corn	445	4.30	103
oat	433	17.0	25.0
canola	410	12.8	32.0
canola protein	362	51.2	7.08
soybean	397	10.7	37.0
alfalfa	350	36.0	10.0
alfalfa protein	226	59.5	3.80

[a] Unidentified native grass species.

Table 2. Properties of Soils Used in This Study[a]

soil	organic C (g kg^{-1})	total N (g kg^{-1})	C/N ratio	pH	sand (g kg^{-1})	clay (g kg^{-1})
Wooster NT[b]	24.6	2.42	10.2	6.03	239	180
Wooster CT	14.6	1.56	9.36	5.63	248	162
Wooster PT	9.78	1.12	8.73	6.13	250	150
Hoytville NT	35.2	3.31	10.6	6.76	205	410
Hoytville CT	21.9	2.36	9.27	7.05	205	411
Hoytville PT	19.4	2.16	8.98	6.17	210	400
Walnut Creek[c]						
Clarion 1	12.1	1.29	9.38	4.76	542	203
Clarion 2	20.1	1.50	13.4	5.33	440	220
Webster	37.2	2.40	15.6	7.59	390	270
Harps 1	32.1	2.61	13.8	7.16	350	280
Harps 2	33.4	2.32	14.3	7.61	320	300
Canesteo	33.6	2.50	13.4	5.60	276	351
Baker[d]						
Webster 1	32.2	2.52	12.8	6.81	398	254
Webster 2	19.9	1.31	15.1	5.42	513	199
Webster 3	18.7	1.43	13.1	6.21	531	161
Okoboji	40.3	3.37	12.0	6.18	104	351
Harps 1	42.1	3.11	13.5	6.47	186	360
Harps 2	33.4	2.32	14.4	7.46	103	433
Clarion 1	12.1	1.29	9.38	7.88	604	145
Clarion 2	20.1	1.50	13.4	4.99	579	206
Zencor 1	14.2	1.10	12.9	5.21	683	147
Zencor 2	12.1	1.29	9.38	7.35	765	76.0

[a] See Materials and Methods for description of analyses used. [b] Wooster and Hoytville continuous corn tillage management, no-tillage, NT; chisel tillage, CT; and plow tillage, PT. [c] Clarion–Nicollet–Webster tillage management for corn was fall chisel with spring field cultivation and for soybean was spring field cultivation. [d] Baker tillage management for corn was fall chisel with spring field cultivation and for soybean was spring field cultivation.

different N fractions (*10*, *12*), colorimetic analysis for total AA content (*10*) and total AS content (*13*), and reverse phase HPLC, gas chromatography (GC), or ion exchange chromatography (IC) with pre- or postcolumn derivation for compositional analysis. Only the HPLC, GC, or IC methodologies can determine the composition of soil AAs as well as a total AA-N content, but soil acid hydrolysis extracts also result in dissolution of organic matter and mineral matter. In addition to organic contamination, considerable amounts of Si^{4+}, Al^{3+}, and Fe^{3+} are the main cause of quantification interferences (*14*), which results in a laborious cleanup procedure before sample derivation for previously published HPLC or GC methods (*14*).

Martens and Frankenberger (*15*) reported that the use of ion chromatography coupled with a new method of amperometric detection (*16*) showed great potential for the determination of AAs from plant exudates present in high salt content plant nutrient solutions. The method was as fast and more sensitive and robust compared with a ninhydrin derivation method and involved acid digestion with a nonoxidizing acid, 4 M methanesulfonic acid (MSA), neutralization of excess acidity, and dilution as the only sample preparation. The use of amperometry as a detection methodology gave the added advantage of detecting the ASs as well as the AAs in a single chromatographic analysis (*15*). The use of MSA has several advantages over 6 M HCl. First, MSA is nonvolatile and thermally stable at elevated temperatures; second, it does not oxidize the S-containing AAs or destroy tryptophan, serine, and threonine; and, third, the use of MSA results in quantitative recovery of AA composition found in purified proteins (*15*, *17*).

The goals of this work were to compare the use of MSA and HCl for accounting of biological organic N, to standardize the conditions for hydrolysis of soil organic N content, and to identify the composition of soil organic N. The standardized conditions were then applied to analyzing the importance of soil organic N composition in a Midwest corn (*Zea mays*)–soybean (*Glycine max*) rotation to soil N cycling.

MATERIALS AND METHODS

Plant and Soil Samples. Properties of the corn, soybean, an unidentified prairie grass, alfalfa (*Medicago sativa*), oat (*Avena sativa*), clover (*Trifoium pratense*), and canola (*Brassica napus*), harvested from overwintered field sites or from glasshouse pots, are listed in **Table 1**. The plant samples analyzed were a mix of above-ground leaf and stem portions (ground through a 1 mm sieve). Selected properties of the 22 soils used are reported in **Table 2**. Experiments chosen were the long-term (36 years) continuous corn tillage (0–5 cm depth) comparison (no-tillage, NT; chisel tillage, CT; and plow tillage, PT) plots at Wooster and Hoytville, OH (*18*, *19*), the long-term (67 years) Baker corn–soybean (0–15 cm depth) management site, Ames, IA (*20*), and a privately managed Clarion–Webster–Nicollet corn–soybean (0–15 cm depth) field (Walnut Creek soils) with experiments conducted by the USDA-ARS National Soil Tilth Laboratory, Ames. IA. The soils were composites of three to five samples collected within a 1 m^2 area and stored moist at 4 °C until quickly air-dried and sieved to pass a 1.0 mm screen. The pH was measured in 0.01 M $CaCl_2$ and soil texture by a pipet method described by Gee and Bauder (*21*). Total organic C and total N were determined by dry combustion with a Perkin-Elmer 2400 C/H/N analyzer (Perkin-Elmer, Inc., Fullerton, CA) (**Table 1**).

Plant Protein Extraction. Total protein N extraction efficiency was measured by extracting plant tissue with MSA at different time intervals coupled with ion chromatography with pulsed amperometric detection of individual AAs (*15*). Briefly, 20 mg of plant tissue in screw-top test tubes (15 × 125 mm) was treated with 2 mL of 4 M MSA, and the samples were autoclaved for 30, 60, or 90 min at 136 °C (112 kPa) or for 16 h at 121 °C (104 kPa). The HCl-treated (2 mL of 6 M HCl) 20 mg of plant tissue was flame sealed in ampules before digestion for 16 h at 121 °C (104 kPa). Following digestion, the samples were titrated to pH 4–5 with 5 M KOH and centrifuged to remove precipitate, and then an aliquot was diluted for analysis. Alfalfa and canola plant residue proteins were extracted by maceration with a 0.10 M Tris-HCl buffer (pH 8.0) with reducing agents and protease inhibitors as described by Gegenheimer (*22*), precipitated in an ice bath with acetone, and recovered by centrifugation.

Soil Organic N Extraction Standardization. To optimize conditions for soil organic N extraction, soils (100 mg) were treated with 2 mL of 4 M MSA, and the samples were autoclaved for 30, 60, or 90 min at 136 °C or for 16 h at 121 °C (104 kPa). After sample cooling, centrifugation, and collection of the supernatant, the residue was washed with two aliquots of 1 mL of deionized (DI) water and centrifuged between each addition, and the three supernatants were combined for AA analysis. In addition, soil samples (100 mg) were also digested with 2 mL of 6 M HCl hydrolyzed under reflux at 110 °C for 12 h as modified from ref *8*. All combined supernatants were titrated to pH 4–5 with 5 M KOH and centrifuged to remove precipitate, and then an aliquot was diluted for analysis. Total organic N recovered from the plant and soil digestions was calculated as AA-N, AS-N, and NH_4^+ released by acid digestion. The NH_4^+ concentration in the digest supernatant was determined by steam distillation (*23*).

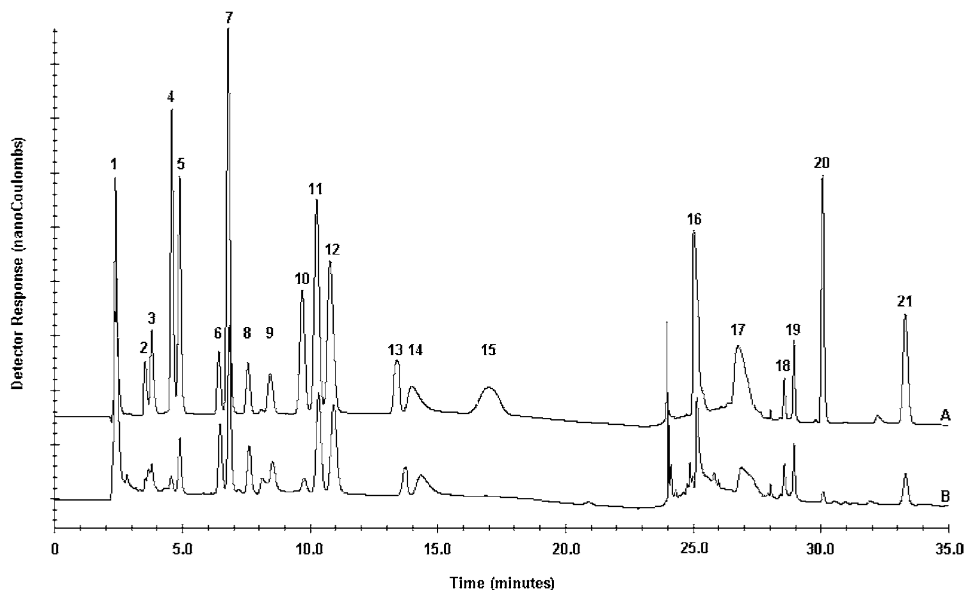

Figure 1. Chromatographic traces of (A) 500 pM amino acid and amino sugar standards and (B) amino acids and amino sugars extracted from oat tissue. Amino acid and sugar identification: 1 = Arg; 2 = ornithine; 3 = Lys; 4 = galactosamine; 5 = glucosamine; 6 = Ala; 7 = Thr; 8 = Gly; 9 = Val; 10 = Hpro; 11 = Ser; 12 = Pro; 13 = Ile; 14 = Leu; 15 = Met; 16 = His; 17 = Phe; 18 = Glu; 19 = Asp; 20 = Cys_2; 21 = Tyr.

Identification of Amino Acids and Amino Sugars. The AAs and ASs released as hydrolysis products of acid digestion were separated on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) ion chromatograph equipped with a 25 μL injection loop and an AminoPac PA10 guard and analytical column (250 mm × 2 mm i.d.). Separation was achieved with a tertiary water, NaOH (5−80 mM), and sodium acetate (125−200 mM) gradient for AAs and ASs (*15*). Pulsed amperometric detection was by a Dionex ED-40 electrochemical detector set in the integrated pulsed mode with a disposable gold working electrode. Jandik et al. (*24*) presented a detailed chapter on the IC gradients and integrated amperometry detection methods employed here for AA and AS separation and detection. The AA standards [AA kit (catalog no. LAA-10) and standard solutions (catalog no. AA-S-18)] were obtained from Sigma Chemical Co., St. Louis, MO, and were used to map retention times and detector response. The chromatographic parameters for the AminoPac column including AA and AS retention times, column capacity factor, and precision and limits of amperometric detection for the method described here were given by Martens and Frankenberger (*15*).

RESULTS AND DISCUSSION

The plant material chosen represents agricultural crops with a range in C/N ratios (**Table 1**). The soils represent long-term tillage experiments based on two Ohio soils (Wooster and Hoytville) and two locations in central Iowa that have been monitored by the USDA-ARS National Soil Tilth Laboratory, Ames IA, each with typical soils of the area. The combination of management and landscape position has resulted in a wide range of soil properties (**Table 2**).

An advantage to the use of ion chromatography with pulsed amperometric detection is that the nonderivatized samples can be selectively analyzed simultaneously for both ASs and AAs (**Figure 1A**). To acquire the same information would require two analysis steps with derivatization methodologies. The two overwintered plant residues, corn and soybean, showed higher concentrations of the ASs galatosamine and glucosamine compared to the fresh plant samples and suggested a high level of microbial activity on plant residue exposed to a soil environment (**Table 3**). Comparison between the traditional 6 M HCl and 4 M MSA found that MSA gave better AA recovery due to the lack of AA degradation and conversion to non-AA compounds such as cysteine to cysteic acid. The chromatographic method showed that MSA digestions quantitatively accounted for the total content and composition of the AA-N (plus NH_4^+ released) from the different plant materials tested (**Table 3**; **Figure 1B**). Several noticeable trends in composition were the high concentrations of Arg present in the oat material and the expected high levels of Glu and Asp in the legumes, clover and soybean. Only the digestion of clover did not result in quantitative recovery of plant total N as AA-N. The clover had been grown in the glasshouse and harvested at an early growth stage, whereas the other legume species, soybean, had been collected from a farm field following overwintering.

Table 3. Amino Acid (AA) and Amino Sugar (AS) Composition of Plant Residue Extracted with Methanesulfonic Acid (MSA) or HCl[a]

	clover (mg g^{-1})		prairie (mg g^{-1})		corn (mg g^{-1})		oat (mg g^{-1})		soybean (mg g^{-1})	
AA-AS	MSA	HCl	MSA	HCl	MSA	HCl	MSA	HCl	MSA	HCl
Arg	6.94	5.23	3.76	3.56	6.51	5.56	26.2	23.2	4.32	4.12
Lys	3.00	2.85	1.02	0.94	0.55	0.46	2.72	2.45	4.28	3.89
Galx	0.00	0.00	0.39	0.12	0.33	0.23	0.17	0.16	1.23	0.97
Glux	0.44	0.14	0.98	0.56	1.54	1.23	0.53	0.51	2.91	2.56
Ala	1.83	1.53	1.13	0.86	0.81	0.62	3.83	3.23	3.80	3.56
Thr	3.39	1.23	1.32	0.87	0.72	0.42	2.37	1.05	3.46	1.36
Gly	0.33	0.20	0.66	0.56	0.26	0.16	2.73	2.54	6.70	6.43
Val	1.49	1.23	1.00	0.98	0.45	0.43	3.62	3.23	6.01	5.56
Hpro	1.48	1.35	0.12	0.12	0.35	0.34	0.31	0.23	1.75	1.23
Ser	1.18	0.55	0.70	0.33	0.29	0.12	2.13	0.79	2.40	0.98
Pro	1.05	0.86	0.26	0.24	0.11	0.09	3.01	2.95	3.47	3.45
Ile	1.32	1.12	0.68	0.45	0.47	0.26	2.54	2.33	4.07	3.56
Leu	3.06	1.23	1.44	1.23	0.79	0.46	4.83	4.23	7.75	7.23
His	1.83	1.65	0.34	0.24	0.68	0.56	1.52	1.23	1.88	1.56
Phe	5.03	4.23	0.79	0.59	0.84	0.82	2.20	2.03	2.25	2.13
Glu	10.5	9.86	1.96	1.78	1.71	1.23	3.52	3.24	4.49	4.23
Asp	10.9	8.65	1.48	1.35	1.84	1.65	2.38	2.18	4.14	4.13
Cys_2	1.08	0.00	0.18	0.00	0.15	0.00	0.20	0.00	0.44	0.00
Tyr	7.52	5.23	0.74	0.34	0.74	0.53	1.34	1.12	3.72	2.98
total AA	62.4	47.1	19.1	15.1	19.3	15.2	67.5	56.7	70.6	59.9
SD	2.31	1.55	3.64	1.66	1.31	0.37	3.21	1.56	2.12	1.23
% total N[b]	23.8	19.8	93.3	67.5	93.2	65.3	93.2	81.2	101	78.5

[a] Methanesulfonic acid (4 M) and HCl (6 M) were used to digest the plant tissue (16 h, 121 °C). Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

Table 4. Amino Acid (AA) and Amino Sugar (AS) Composition of Plant Residue and Plant Proteins Extracted with Methanesulfonic Acid (MSA) with Two Digestion Times[a]

AA-AS	canola (mg g^{-1})		canola protein (mg g^{-1})		alfalfa (mg g^{-1})		alfalfa protein (mg g^{-1})	
	MSA1	MSA2	MSA1	MSA2	MSA1	MSA2	MSA1	MSA2
Arg	21.5	2.20	45.2	4.54	20.4	3.14	32.9	5.34
Lys	2.76	0.43	15.1	1.95	14.1	1.46	30.0	0.95
Galx	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glux	0.00	0.00	0.00	0.00	0.73	0.00	0.00	0.00
Ala	2.96	0.62	9.64	2.16	16.1	1.80	17.3	1.96
Thr	1.95	0.33	10.5	1.46	9.25	0.98	23.9	1.23
Gly	0.90	0.83	20.7	3.14	4.32	2.76	31.3	1.83
Val	2.89	0.40	22.9	1.70	7.11	1.34	32.3	1.23
Hpro	1.12	0.13	3.22	0.66	1.20	0.12	3.44	0.23
Ser	0.73	0.32	4.65	0.89	4.23	1.05	8.45	0.99
Pro	0.20	0.30	4.04	0.71	2.28	0.80	7.50	1.36
Ile	1.54	0.35	14.3	1.71	6.82	1.08	18.4	1.23
Leu	2.91	0.98	28.6	4.43	9.50	2.53	43.3	3.36
His	1.15	0.22	6.11	0.99	2.57	0.66	13.6	0.78
Phe	2.56	0.55	8.61	2.31	7.50	1.23	15.3	1.56
Glu	4.86	1.06	22.8	4.46	14.1	2.80	35.9	3.75
Asp	3.65	0.72	15.3	2.76	17.3	2.10	38.8	9.78
Cys_2	0.60	0.10	2.52	0.20	0.76	0.18	3.16	0.54
Tyr	2.61	0.41	17.2	1.70	6.50	1.00	25.8	3.52
total AA	54.9	9.95	251	36.1	145	25.1	381	36.6
SD	2.91	1.65	5.64	3.56	4.31	3.37	8.21	4.16
% total N[b]	93.1	14.6	86.3	11.9	71.7	13.5	94.1	11.2

[a] Plant material was treated with 2 mL of 4 M MSA and autoclaved for 16 h (MSA1) at 121 °C (104 kPa) or for 90 min (MSA2) at 136 °C (112 kPa). Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

Crawford et al. (*25*) reported two different fixed N transport mechanisms in legume species. The first pathway includes species such as soybean and clover and involves the export of fixed N from the nodules as ureides before conversion to asparagine (Asn) and glutamine (Gln), and the second pathway, found in alfalfa and peas, involves the transport of Asn and Gln from the nodules. The lack of clover AA recovery suggests that the majority of the N content at the early growth stage of the harvested clover was present as the non-AA ureide compounds allantoic acid and allantoin (*25*), whereas in the mature vegetation (soybean), the total N content was present as AAs. During acid digestion, Asn and Gln are degraded and recovered as Asp and Glu.

Proteins have been found to require at least 16−20 h of autoclave digestion to account for the different strengths of the N linkages (*26*). Comparison of a shorter digestion time (1.5 h) with an elevated temperature (136 °C) was made to the standard 16 h digestion with the 4 M MSA (**Table 4**) to determine if the extraction process could be shortened. The results show that the shorter time was not adequate for release of the individual amino compounds from plant tissue. The 16 h MSA hydrolysis conditions were effective for releasing the AA composition from the plant and the extracted plant proteins. The failure to completely hydrolyze polymeric AAs with the shorter digestion time will limit the AA methodology for plant protein analysis to laboratories equipped with programmable autoclaves capable of doing the extended digestion times.

No studies have been published that determine optimum conditions for release of AAs from soil organic matter. The failure to develop optimum conditions is no doubt due to the rather poor recovery of only 30−40% of the total soil N as AAs with wet chemical methods. Until recently, all soil AA digestions in our laboratory were conducted with a standard 16 h hydrolysis method as employed for the previously described plant tissue analysis with AA recovery comparable (**Table 5**; 36.5% recovery, $n = 3$) to the published HCl soil AA recovery (*2*). Due to an extensive downtime of our laboratory's programmable autoclave, we investigated the use of shorter times with higher temperatures for recovery of soil AA-N in a small tabletop autoclave. Unexpectedly, we found much higher recoveries of soil AA-N with the shorter times (**Table 5**) that were not found with the same experiments with the plant

Table 5. Amino Acid (AA) and Amino Sugar (AS) Composition of Wooster Soils Extracted with Methanesulfonic Acid (MSA) at Different Times and Temperatures ($n = 2$)[a]

AA-AS	Wooster NT (mg g^{-1})				Wooster CT (mg g^{-1})				Wooster PT (mg g^{-1})			
	16 h	0.5 h	1.0 h	1.5 h	16 h	0.5 h	1.0 h	1.5 h	16 h	0.5 h	1.0 h	1.5 h
Arg	0.57	0.59	0.72	0.75	0.24	0.39	0.51	0.61	0.31	0.29	0.33	0.40
Lys	0.24	0.51	0.55	0.53	0.11	0.30	0.34	0.36	0.18	0.21	0.21	0.24
Galx	0.28	0.79	0.84	0.77	0.07	0.38	0.41	0.40	0.12	0.25	0.25	0.25
Glux	0.52	1.09	1.23	1.19	0.22	0.65	0.77	0.84	0.29	0.42	0.46	0.50
Ala	0.17	0.37	0.51	0.57	0.17	0.22	0.30	0.34	0.11	0.15	0.18	0.19
Thr	0.15	0.27	0.37	0.44	0.07	0.13	0.22	0.26	0.07	0.08	0.13	0.15
Gly	0.18	0.36	0.56	0.63	0.12	0.21	0.35	0.39	0.11	0.14	0.23	0.26
Val	0.18	1.09	1.72	1.88	0.10	0.91	1.68	1.99	0.09	0.80	1.37	0.77
Hpro	0.02	0.09	0.14	0.13	0.01	0.07	0.07	0.11	0.01	0.05	0.05	0.01
Ser	0.09	0.12	0.24	0.23	0.08	0.14	0.15	0.19	0.02	0.05	0.06	0.07
Pro	0.06	0.22	0.20	0.21	0.05	0.03	0.12	0.20	0.04	0.07	0.08	0.07
Ile	0.09	0.08	0.15	0.17	0.05	0.03	0.08	0.09	0.05	0.02	0.03	0.05
Leu	0.20	0.15	0.30	0.33	0.11	0.06	0.14	0.16	0.08	0.03	0.05	0.07
His	0.08	0.02	0.02	0.04	0.10	0.13	0.13	0.13	0.09	0.10	0.10	0.10
Phe	0.09	0.05	0.04	0.04	0.03	0.02	0.03	0.03	0.01	0.02	0.03	0.02
Glu	0.15	0.52	0.58	0.52	0.14	0.29	0.37	0.37	0.11	0.23	0.26	0.30
Asp	0.07	0.62	0.62	0.59	0.14	0.35	0.39	0.42	0.07	.027	0.29	0.35
Cys_2	0.08	0.08	0.08	0.08	0.00	0.04	0.04	0.04	0.01	0.02	0.02	0.03
Tyr	0.07	0.16	0.16	0.15	0.01	0.08	0.08	0.08	0.03	0.05	0.05	0.06
total AA	3.20	7.35	9.10	9.39	1.78	4.38	6.11	6.85	1.80	3.21	4.18	3.83
SD	0.96	0.65	0.61	2.24	0.33	0.37	0.70	0.14	0.18	0.06	0.87	0.09
% total N[b]	31.9	63.8	78.2	84.6	34.0	66.5	89.4	92.6	43.5	69.3	91.6	87.6

[a] Soils (100 mg) were treated with 2 mL of 4 M MSA and autoclaved for 30, 60, or 90 min at 136 °C or for 16 h at 121 °C (104 kPa). NT, no-tillage; MT, chisel; PT, plow tillage management. Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

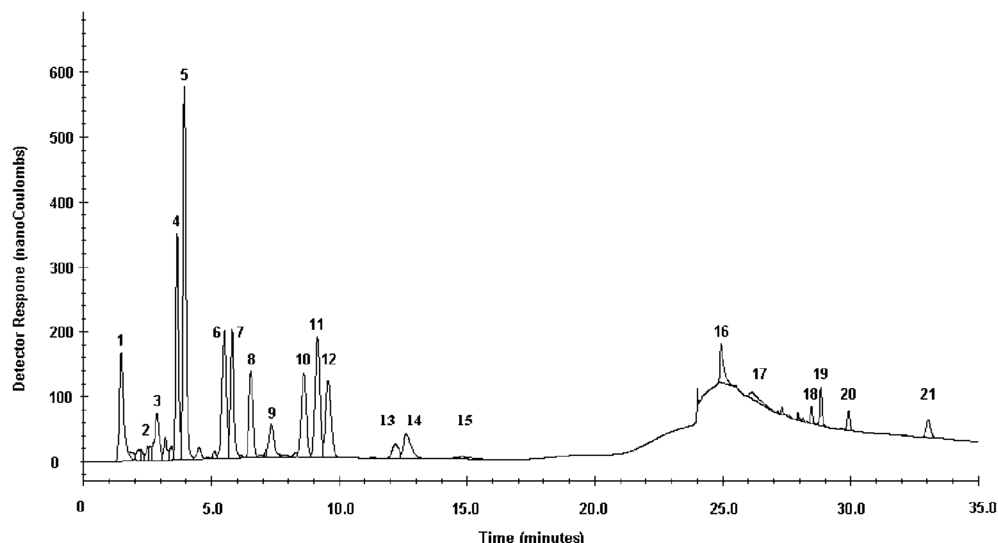

Figure 2. Chromatographic trace of amino acids and sugars extracted from a Wooster soil under no-tillage management with 2 mL of methanesulfonic acid (1.5 h, 136 °C, 112 kPa). Peak identities are listed in **Figure 1**.

material (**Table 4**). The chromatographic result for the 1.5 h/136 °C MSA digestion of a silty loam Wooster soil with IC separation and amperometric detection is shown in **Figure 2**. The AA-N concentration combined with the recovered NH_4^+ released from the AAs and soils by the shorter acid digestions accounted for nearly 90% ($n = 3$) of the total soil N content in the 1.5 h digestions of the Wooster soils from different tillage management (**Table 5**). In comparison, a HCl digestion from the Wooster soils averaged 42.3% AA-N ($n = 3$) or a 2-fold MSA extraction efficiency increase (data not shown).

The decreased digestion time resulted in an increased soil AA release that was nearly the same for the majority of AA concentrations recovered, when compared as a ratio of individual AA to the total AA release, except for Val, which showed nearly a 5-fold increase with increased digestion time (**Figure 3A**). Beavis and Mott (*6*) reported using the relative soil AA concentration (individual AA concentration/total AA concentration) as a "fingerprint" to determine differences in the pattern of AA distribution relative to a specific soil management. A similar AA fingerprint pattern was obtained for the majority of AAs when the AA ratio from the Wooster soil under different tillage management were compared, even with a 3-fold difference in organic C content (**Figure 3B**), suggesting that the sequestration or mineralization of AA concentration in the soils was uniform for all of the AAs. The work is consistent with the results of Keeney and Bremner (*27*), who utilized acid digestion and steam distillation techniques to first report the pattern of uniform sequestration or mineralization of soil AAs in response to soil management pressure.

When AA hydrolysis experiments were expanded to a similar set of tillage treatments for Hoytville clay loam soils, the results were different from the results of the silty loam Wooster soil. The Hoytville soil released the maximum AA-N with the 30 min autoclave hydrolysis (**Table 7**) compared with maximum AA-N release from the 1.5 h Wooster digestions (**Table 6**). The optimum hydrolysis time of 0.5 h gave an average 81% AA-N recovery (AA-N + NH_4^+) versus the average 1.5 h autoclave hydrolysis time recovery of 64% for the Hoytville soils. The Hoytville results suggest that reactions of released AAs during digestion with the soil clay minerals may be partly responsible for reduced recovery of AAs from soil hydrolysis. The results from the two soils suggest that a soil standardization test would be required for maximum recovery of AA-N from soils

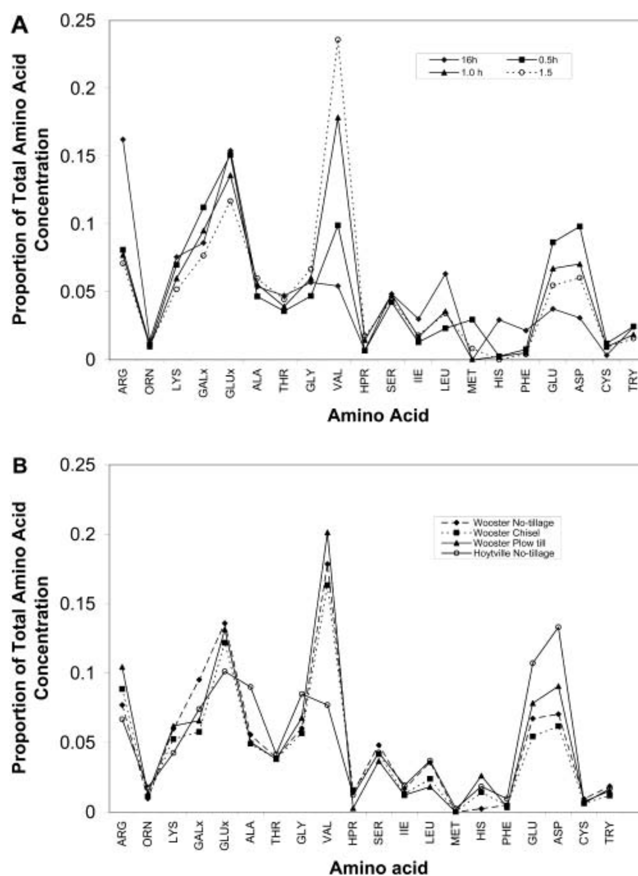

Figure 3. Relative amino acid concentrations of (A) the Wooster soil under no-tillage digested with 4 M methanesulfonic acid at 16 h (121 °C, 104 kPa) or at 0.5, 1.0, and 1.5 h (136 °C, 112 kPa) and (B) the different Wooster tillage managements in comparison with the Hoytville no-tillage. Galx, galactosamine; Glux, glucosamine.

originating from different parent materials. Application of the AA fingerprint pattern for the alluvial Hoytville clay loam soil found similarities to several of the AA concentrations in the silty loam Wooster soil AA fingerprint pattern (**Figure 3B**), but the majority of the Hoytville AA ratios were different from the ratios from the Wooster soil. The data suggest that the fingerprint application as proposed (*6*) may be useful for

Table 6. Amino Acid (AA) and Amino Sugar (AS) Composition of Hoytville Soils Extracted with Methanesulfonic Acid (MSA) at Different Times and Temperatures ($n = 2$)[a]

	Hoytville NT (mg g^{-1})				Hoytville CT (mg g^{-1})				Hoytville PT (mg g^{-1})			
AA-AS	16 h	0.5 h	1.0 h	1.5 h	16 h	0.5 h	1.0 h	1.5 h	16 h	0.5 h	1.0 h	1.5 h
Arg	1.47	1.02	0.74	0.83	0.89	0.52	0.45	0.59	0.98	0.36	0.32	0.46
Lys	0.54	0.65	0.41	0.59	0.36	0.25	0.21	0.41	0.37	0.21	0.18	0.33
Galx	0.53	1.13	0.89	0.87	0.23	0.53	0.44	0.47	0.22	0.38	0.33	0.35
Glux	0.97	1.55	1.33	1.32	0.42	0.76	0.70	0.74	0.38	0.52	0.48	0.51
Ala	0.53	1.38	0.59	0.50	0.26	0.51	0.30	0.30	0.24	0.37	0.23	0.25
Thr	0.35	0.64	0.39	0.43	0.14	0.22	0.17	0.23	0.13	0.15	0.12	0.18
Gly	0.62	1.30	0.64	0.63	0.32	0.49	0.3	0.39	0.30	0.34	0.23	0.33
Val	0.63	1.18	0.58	0.55	0.43	0.45	0.32	0.36	0.31	0.28	0.26	0.29
Hpro	0.06	0.19	0.08	0.08	0.02	0.05	0.04	0.04	0.03	0.04	0.04	0.04
Ser	0.48	0.33	0.19	0.23	0.14	0.13	0.11	0.12	0.12	0.08	0.06	0.08
Pro	0.20	0.33	0.21	0.22	0.11	0.13	0.08	0.13	0.12	0.09	0.07	0.09
Ile	0.26	0.29	0.18	0.17	0.10	0.09	0.07	0.09	0.09	0.05	0.05	0.06
Leu	0.11	0.57	0.31	0.34	0.09	0.16	0.12	0.15	0.08	0.09	0.07	0.09
His	0.17	0.28	0.14	0.13	0.09	0.11	0.07	0.08	0.01	0.07	0.02	0.05
Phe	0.35	0.15	0.12	0.11	0.16	0.04	0.06	0.07	0.02	0.02	0.03	0.04
Glu	0.24	1.64	0.76	0.58	0.19	0.58	0.40	0.35	0.13	0.45	0.29	0.25
Asp	0.19	2.04	1.03	0.76	0.20	0.84	0.58	0.52	0.17	0.69	0.44	0.40
Cys_2	0.02	0.10	0.06	0.07	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02
Tyr	0.30	0.26	0.18	0.20	0.11	0.10	0.09	0.10	0.09	0.07	0.06	0.06
total AA	9.25	15.3	9.24	8.72	4.40	6.44	4.92	5.21	3.87	4.60	3.54	3.93
SD	1.23	0.54	1.82	0.29	0.89	0.36	0.23	0.25	0.36	0.11	0.04	0.11
% total N[b]	50.9	88.7	59.5	60.4	43.0	77.0	58.0	59.6	44.5	76.4	62.5	70.5

[a] Soils (100 mg) were treated with 2 mL of 4 M MSA and autoclaved for 30, 60, or 90 min at 136 °C or for 16 h at 121 °C (104 kPa). NT, no-tillage; MT, chisel; PT, plow tillage management. Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

Table 7. Amino Acid (AA) and Amino Sugar (AS) Composition of Walnut Creek Soils Sampled in April 1998 Extracted with Methanesulfonic Acid (MSA)[a]

	soils (mg g^{-1})					
AA-AS	Clarion 1	Clarion 2	Webster	Harps 1	Harps 2	Canesteo
Arg	0.71	0.94	0.97	1.08	1.03	1.04
Lys	0.63	0.87	0.70	0.50	0.72	0.83
Galx	0.41	0.60	0.48	0.57	0.47	0.63
Glux	0.83	1.07	0.67	0.86	0.61	1.07
Ala	0.32	0.38	0.57	0.58	0.60	0.46
Thr	0.19	0.22	0.32	0.33	0.33	0.27
Gly	0.33	0.42	0.58	0.62	0.59	0.52
Val	0.33	0.38	0.24	0.34	0.31	0.36
Hpro	0.06	0.07	0.05	0.07	0.05	0.05
Ser	0.09	0.16	0.22	0.26	0.23	0.20
Pro	0.11	0.09	0.10	0.11	0.11	0.12
Ile	0.07	0.09	0.14	0.17	0.15	0.12
Leu	0.14	0.18	0.26	0.32	0.28	0.30
His	0.12	0.22	0.14	0.09	0.13	0.08
Phe	0.01	0.02	0.02	0.03	0.02	0.02
Glu	0.59	0.50	0.68	0.68	0.70	0.59
Asp	0.78	0.70	1.47	1.16	1.50	0.93
Cys_2	0.08	0.04	0.05	0.06	0.04	0.05
Tyr	0.07	0.07	0.10	0.13	0.12	0.07
total AA	5.98	6.98	7.72	8.44	8.12	7.69
SD	0.17	0.37	0.11	0.37	0.31	0.05
% total N[b]	88.1	89.9	85.6	85.8	86.6	87.8

[a] Soils (100 mg) were treated with 2 mL of 4 M MSA and autoclaved for 60 min at 136 °C (112 kPa). Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

understanding changes in the AA concentrations from different soils. The results counter the conclusions of Schulten and Schnitzer (*2*), who found little variability in soil AA composition and dismissed the usefulness of soil AA concentration as an indicator of biological properties.

The potential impact of clay content on differences in the hydrolysis time results found for the Wooster and Hoytville soils was further tested on a Clarion−Webster−Nicollett soil series from a field in central Iowa that ranged in clay content from 203 to 351 g of clay kg^{-1} of soil. The results given in **Table 7** were based on the 1.0 h autoclave time that was optimum for each soil tested, regardless of the clay content. The 1.0 h digestion resulted in an average AA- + NH_4^+-N recovery that was 87.3% of the total N content of the soils. The results confirm the need for a preliminary test to determine the optimum hydrolysis time required for different soil types. The difference in clay content noted with the Wooster and Hoytville comparison, not apparent with the Iowa soils, may be due to differences in parent material. The Iowa soils were derived from the same parent material and vegetation, whereas the Ohio soils were not formed under the same conditions.

The high recovery of total N content as AA-N [MSA 85.9%, $n = 12$, compared with 46.6% for HCl (data not shown)] from the Wooster, Hoytville, and Clarion−Webster−Nicollett soils (**Tables 5−7**) with a wide range in soil properties confirms that the majority of soil N content is composed of AAs, ASs, and NH_4^+ with a documented composition. Although pyrolysis methods have identified certain heterocyclic N-containing compounds as genuine components of the soil non-AA-N pool, pyrolysis has also recognized that other heterocyclics identified are possible reaction products of proteinaceous materials (*28*). The digestion results with plant tissue and extracted proteins that required at least 16 h of digestion suggest that the soil organic N is not composed of large intact proteins as found in plant tissue. The soil organic N due to the presence of many protease enzymes may reflect more of a peptide nature. Leinweber and Schulten (*5*) and others (*29*) have found evidence of peptide materials in soil extracts. Although the use of 6 M HCl has resulted in a lower recovery of AA-N, recent work by Leinweber and Schulten (*28*) has shown that a significant portion of the nonhydrolyzable or non 6 M HCl extracted soil N was composed of AAs that were bound at reactive surfaces and showed evidence of being composed of peptides.

Table 8. Amino Acid (AA) and Amino Sugar (AS) Composition of Baker Soils Sampled in April 1997 Extracted with Methanesulfonic Acid (MSA)

	soils (mg g^{-1})									
AA-AS	Webster 1	Webster 2	Webster 3	Okoboji	Harps 1	Harps 2	Clarion 1	Clarion 2	Zencor 1	Zencor 2
Arg	0.94	0.84	0.70	0.97	0.83	0.78	0.32	0.35	0.64	0.30
Lys	0.66	0.61	0.65	0.73	0.60	0.57	0.26	0.31	0.44	0.17
Galx	0.65	0.57	0.60	0.72	0.70	0.67	0.26	0.31	0.51	0.17
Glux	0.96	0.98	0.91	1.17	1.02	1.03	0.43	0.61	0.85	0.27
Ala	1.09	0.85	0.96	1.11	1.06	1.17	0.42	0.42	0.96	0.23
Thr	0.37	0.27	0.31	0.35	0.39	0.43	0.15	0.14	0.25	0.07
Gly	0.67	0.52	0.61	0.65	0.53	0.60	0.28	0.26	0.37	0.15
Val	0.26	0.21	0.23	0.30	0.30	0.34	0.13	0.12	0.15	0.07
Hpro	0.28	0.23	0.25	0.27	0.28	0.33	0.15	0.13	0.06	0.07
Ser	0.19	0.09	0.09	0.12	0.13	0.15	0.04	0.06	0.12	0.03
Pro	0.10	0.07	0.10	0.10	0.09	0.10	0.05	0.04	0.10	0.02
Ile	0.13	0.09	0.10	0.12	0.15	0.15	0.04	0.05	0.08	0.03
Leu	0.22	0.16	0.19	0.22	0.26	0.28	0.09	0.08	0.14	0.06
His	0.16	0.09	0.14	0.08	0.12	0.08	0.07	0.08	0.13	0.06
Phe	0.24	0.13	0.14	0.23	0.20	0.06	0.10	0.10	0.17	0.09
Glu	0.82	0.55	0.68	0.85	0.57	0.78	0.48	0.41	0.58	0.25
Asp	1.45	0.82	0.79	1.02	0.69	0.72	0.56	0.42	0.65	0.31
Cys_2	0.08	0.05	0.04	0.06	0.03	0.04	0.03	0.03	0.04	0.02
Tyr	0.10	0.07	0.09	0.10	0.10	0.11	0.06	0.04	0.07	0.03
total AA	9.66	7.48	7.94	9.62	8.47	8.30	4.04	4.12	6.56	2.47
% total N[b]	91.8	92.9	86.4	78.5	78.3	63.8	87.9	96.2	87.0	89.3

[a] Soils (100 mg) were treated with 2 mL of 4 M MSA and autoclaved for 60 min at 136 °C (112 kPa). Galx, galactosamine; Glux, glucosamine. [b] Percentage total N recovered was determined by summation of AA-, AS-, and NH_4^+-N measured in the digests dividing the total N content determined by dry combustion.

To determine if the proposed methodology was sufficiently sensitive to determine seasonal changes in soil AA content, a corn−soybean rotation management with the Baker soils was analyzed in the spring and fall of the 1997 and 1998 growing seasons (soybean grown in 1996 and 1998, corn grown in 1997) to determine AA- and AS-N before planting and following harvest. The AA and AS composition and total AA-N as AA, AS, and NH_4^+ content from the Baker soils following the soybean crop (1996 season) sampled in May 1997 are given in **Table 8**. The results showed an average N recovery of 90.2% of total N content from the soils except for the Okoboji and two Harps soils, which showed an AA-N recovery average of 73.5%. The decrease in AA-N extraction effectiveness by MSA from the three soils compared with the remaining seven soils from the field may be due to the periodic flooding of these three topographically low soils. The three soils are present at the lowest elevation in the field (*30*), and the two Harps soils did not yield a soybean crop in 1996 or 1998 due to spring flooding. The Okoboji soil also had reduced 1998 soybean yields due to late spring water ponding. A longer digestion time (16 h) did not result in additional AA recovery, so the presence of protein-N did not account for the failure to recover the AA-N (data not presented). At present few data are available documenting the forms of organic N in soils that are subjected to periodic seasonal flooding, but a hypothesis has been discussed for abiotic nitrate immobilization (*31*). For the abiotic N immobilization to occur, three factors need to be active: first, Fe or Mn minerals to provide the reduction of NO_3^-; second, anaerobic microsites to promote favorable redox conditions; and, third, abundant DOC for immobilization of N. In the Harps and Okoboji soils subject to periodic water ponding, all three conditions would be present for abiotic N immobilization.

The Baker soils were analyzed for the 1997 and 1998 growing seasons that covered a full cycle in a corn−soybean rotation, and analyses found an average ($n = 6$ soils) decrease in AA-N of 315 kg of N ha^{-1} (±264 kg) during the corn-growing season (**Figure 4**). Although the change in soil AA concentration is graphically masked by the high AA concentration, the 1997 N decrease was countered with an average ($n = 6$ soils) N increase during the soybean-growth year of 340 kg of N ha^{-1} (±214 kg). Application of the AA fingerprint interpretation suggests that the majority of the individual AAs from the Baker soils increase and decrease in the same ratio with the total AA content as shown for the Wooster soils and first documented by ref *27*. The fingerprint application is shown in **Figure 5** for the Webster 1 soil analyzed in May and September of 1997 until May 1999

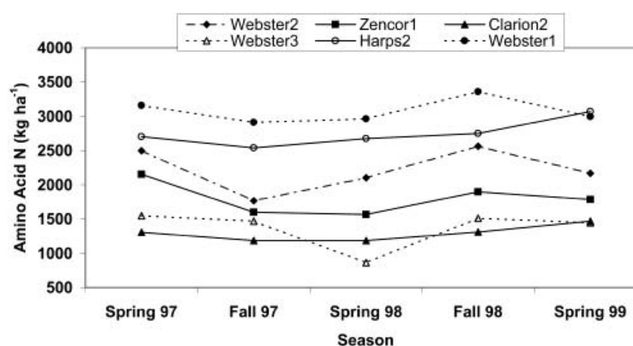

Figure 4. Cycling of the total amino acid-N and sugar-N content in the Baker soils from May 1997 through May 1999. Soybean was grown in 1996 and 1998, and corn was grown in 1997.

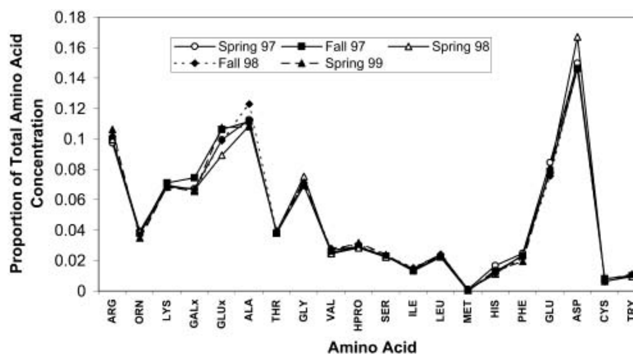

Figure 5. Relative amino acid concentrations of the Baker Webster 1 soil measured from May 1997 through May 1999. Galx, galactosamine; Glux, glucosamine.

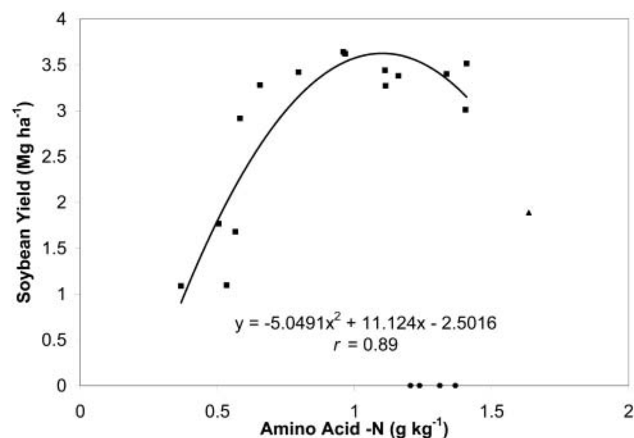

Figure 6. May amino acid-N and sugar-N content relationship with the previous soybean yields in the Baker soils for spring 1997 and 1999. The triangle represents the Okoboji soil, which had experienced reduced plant density due to flooding in June 1998. The solid circles represent the two Harps soils, which experienced prolonged flooding in 1996 and 1998 with complete yield loss. Results from the Harps (1996 and 1998) and Okoboji (1998) soils were not included in the data set.

and suggests that the N credit given for soybean in a corn−soybean rotation may be due to the increase in the soil AA pool.

Recent research by Mayer et al. (*32*) has noted that the influx of N may be due to a large rhizodeposition of N during the growing season from grain legumes. They (*32*) reported that although about two-thirds of the total added ^{15}N assimilated by legumes was present in the grain, the N rhizodeposition constituted 79−85% of the below-ground N at plant maturity with the remaining ^{15}N present as identifiable root biomass. It is apparent from **Figure 4** that the relative AA-N decrease or increase response is dependent on soil type, but all soils showed the decrease in soil AA-N during corn growth and the increase in soil AA-N following the soybean year. The soil AA-N content following soybean growth in 1996 and 1998 followed a trend that higher soybean yield resulted in higher soil AA content (**Figure 6**). Green and Blackmer (*33*) reported that much research documents the decreased N fertilizer requirements for corn after soybean, and Meese (*34*) found that the decreased N requirement during a 6 year study varied greatly from year to year and could range from −245 to 228 kg of N ha^{-1}, but averaged a nitrogen fertilizer decrease of positive 60 kg ha^{-1}. Green and Blackmer (*33*), utilizing ^{15}N additions, concluded that the decreased need for N fertilizers following soybean was due to the timing and extent of N immobilization of soybean residue (fall of growth season) compared with immobilization of corn residue N (following spring). Although the work presented here does not evaluate the timing of the AA-N availability, the data document the important change in the cycling of AAs in soils subjected to corn−soybean rotations and that the increased AA-N content of the soils may be due to a combination of increased rhizodeposition coupled with faster mineralization of soybean residue N following harvest.

This study documents that the majority of the total N content in most well-drained soils is composed of AAs and ASs (plus NH_4^+ released), and the study found a MSA recovered N/total N content ratio of 85.6% for the 22 soils studied. The failure of HCl to completely hydrolyze the organic N forms in soil and the degradative effects of prolonged digestions on soil AA recovery by both MSA and HCl have limited our understanding of soil AAs and their role in crop production. The robust nature of the methodology to determine the soil AA and AS concentrations in a single chromatographic analysis can provide detailed information on the cycling of soil organic N pools that occur during a single growing season and will provide researchers with a valuable tool for further research on N transformations in soils.

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