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SOIL AMENDMENTS DELINEATE AMINO ACID BIOMARKERS MODULATING MACRO-AGGREGATION AND CARBON STABILIZATION

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Utilization of organic matter and synthetic fertilizers in soil generally influences the diversity and dynamics of microbial mass bearing the amino acids, which contain carbon as well as nitrogen being stable components of soil organic matter. This long-term field study initiated in 1989 compared the sole and collective use of compost and NPK fertilizers for exploring microbial amino acids as signatures of carbon turnover in soil exhibited by macro-aggregates formation. Treatments in quadruplicated randomized complete block design experiment were: whole N from organic manure (OM); half N from organic manure + half N from inorganic fertilizer (HOM); mineral NPK fertilizers (NPK), NP, NK, PK, and no fertilizer (control) applied to raise wheat and maize continuously. Classified as calcareous Fluvo-aquic soil (Aquic Inceptisol) and located in Fengqiu County, Henan province, North China Plain, it has sandy loam texture and average pH 8.2. After 23 years of fertilization treatments (in 2012), randomly drawn soil samples from 0–20 cm profile were subjected to various physico-chemical and biochemical determinations. Contents of majority amino acids, viz., arginine, asparagine, aspartic acid, cystine, glutamic acid, leucine, lysine, methionine, proline, and tyrosine were statistically higher under OM and HOM compared to NPK and control treatments. Among the mineral fertilizers, N and P addition but not K mainly contributed to amino acid synthesis / residues in soil; more importantly the cysteine, phenylalanine and serine. Quantitatively increased amino acids positively correlated with improved SOC content that promoted the formation and count of macro-aggregates in soil. These results suggest that accrual of organic and microbial residues largely the amino acids associated with soil micro-aggregates enhance the carbon sequestration in surface soil concurrent with an increased formation and stability of macro-aggregates.
Keywords: Organic matter, microbial residues, soil aggregate formation, carbon sequestration, soil structure.

INTRODUCTION

Current scenario of global climate change owing to increased amounts of greenhouse gases (GHG) principally the carbon (C) and nitrogen (N) derivatives in the atmosphere, threatens several interrelated facets of human subsistence (Shahzad *et al.,* 2017). It lays the foremost emphasis on C-stabilization in agricultural soils representing the largest terrestrial organic C pool - 3 times of CO_2 in the atmosphere and 240 times the fossil fuel emissions yearly (Ciais *et al.,* 2014). Therefore, soil organic carbon (SOC) sequestration - fixing of long-lived C pools in soils for long-term, is an imperative mitigation strategy for climate change (Nawaz *et al.,* 2017), since adding a few percent more to the net C storage in soil embodies a substantive C sink potential (Paustian *et al.,* 2016; Ghosh *et al.,* 2018). As well, SOC in the form of organic matter is crucial in sustaining the essential soil functions, for instance, plant nutrient dynamics and so the soil productivity (Liu *et al.,* 2017). Further, it influences soil structure and properties by contributing to the development of steady aggregates (Zhang *et al.,* 2014). Therefore, total soil organic matter (SOM) is among the most often anticipated soil quality markers, followed by total nitrogen, available phosphorus, water storage, bulk density, cation exchange capacity and microbial biomass etc., all mostly related to SOC (Bünemann *et al.,* 2018).

Well-structured soil mainly ascribed by stable architectural arrangement of soil particles and pore size therein, emerges from aggregation mediated by SOM, microorganisms and plant roots, collectively (Six and Paustian, 2014; Yu *et al.,* 2015). Principal contributing parent materials of SOC or humus are the residues and exudates of plant (primary) and microbial (secondary) source constituting a mixture of individual molecules (monomeric sugars, amino acids), and polymeric molecules (protein, cellulose, lignin) bound together (Kögel-Knabner, 2017). Generally, soil structure development involves clustering of organic and mineral particles (adhered through microbial and root exudates) to form micro-aggregates (53-250 µm) as building blocks contained within stable macro-aggregates (>250 µm), enmeshed by fungal hyphae, earthworms and roots with concomitant C stabilization (Vogel *et al.,* 2014). It reflects that SOM stabilization in soil aggregates could be the main mechanism fostering C-sequestration in soil.

Most of the C or N in plant residues rapidly assimilates into microbial biomass through decomposition, concomitantly forming microbial residues that become stable component of the SOM, largely related to SOM turnover (Liang *et al.,* 2011). Microbial necromass embodies a larger SOM pool, also serving as the time-integrated index of microbes' composition having slower mineralization rate compared to their living biomass (Van-Groenigen *et al.,* 2010). Interestingly, greater bioavailability or addition of N renders positive influence on the stabilization of fresh microbial residues by inhibiting mineralization and transformation of original organic residues-N into microbial residues over long time (Zhang *et al.,* 2015).

Land application of manures and mineral fertilizers alters the SOC accumulation varied with the input source of organic C (Ding *et al.,* 2013). Longer use of manure considerably increases organic C level in soil aggregates, which eventually endorses the macro-aggregation (Yu *et al.,* 2015). However, boost in pore-filling organic matter mainly results from an increase of organic carbon in micro-aggregates (Zhuang *et al.,* 2008). Higher application rates of manure promote soil aggregation with greater SOC storage that correlate with improved microbial residues-mediated aggregate stability (Ding *et al.*, 2015). Microbial residues (i.e., necromass) have relatively longer residence time in soils than their parent plant residues and organic manures (Throckmorton *et al.,* 2015).

Incorporation of crop straw improves soil structure and SOC in macro-aggregates (Zhang *et al.,* 2018). Compost and fertilizer enhance SOC accumulation differently, greater in compost-amended soil primarily attributable to macro-aggregation (Yu *et al.*, 2015). Stabilized humic substances contain far greater amounts and diversity of amino acids (4-6 times higher N) than that of amino sugars therein (Ding *et al.,* 2001). Microbial residues from both bacteria and fungi after manure application and its transformation in cultivated soil accumulate in macro-aggregates (Ding *et al.,* 2015). Amino acids are among the major sources for both C and N in soil; thus, signify a vital link between C and N cycles. In this context, measurement of amino acid contents could be useful to assess microbial contribution to soil aggregation being regarded a strong indicator of C and N stabilization under variable nutrient management practices (Ding *et al.,* 2013).

Combining the soil aggregation with amino acid biomarker analysis could elucidate that how and to what extent the microbial-derived residues in response to differential manure / fertilizer (N, P and K) inputs influence the C-sequestration in these soil microhabitats. This long term field study (launched in 1989) in North China compares the impact of organic and mineral fertilizers on soil macro-aggregation and C-stabilization as related to amino acid composition.

MATERIALS AND METHODS

Study site**:** The present study is part of a long-standing experiment in field commenced on September 1989 at the Fengqiu State Key Agro-ecological Station, Fengqiu County, China (35°00′N, 114°40′E). Wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were grown there in winter and summer, respectively, as test crops. During the last 30 years, air temperature averaged 13.9°C, with monthly values of minimum and maximum correspondingly as 1.0°C (January) while 27.2°C (July). Annual precipitation was 615 mm, being two-third between June and September. Soil of this area has developed on the alluvial sediments of the Yellow River with taxonomic class of Aquic Inceptisol as per USDA soil classification system. Before initiation of this continuing study, the field had been cultivated with similar cropping system for approximately 50 years. Soil was sandy loam textured having 52% sand, 33% silt and 15% clay, with pH 8.65, and contained 4.48 and 0.43 g kg^{-1} organic C and total N, respectively, prior to the commencement of research.

Experimental**:** This field experiment comprised of the treatments: full N from organic manure (OM); half N from organic manure + half N from inorganic fertilizer (HOM); full NPK (nitrogen-phosphorus-potash) from inorganic fertilizer; only inorganic NP fertilizer; only inorganic PK fertilizer; only inorganic NK fertilizer; and no manure or fertilizer (control). These treatments were laid out under randomized complete block design comprising four replicates with each individual plot size of 9.5 m × 5 m. The N was applied @ 150 kg N ha^{-1} to both crops in two split doses as basal and supplemental fertilizers, viz., 60+90 kg N ha^{-1} to maize and 90+60 kg N ha^{-1} to wheat in the plots treated with NPK, NP and NK. For HOM, in maize the 75 kg N ha^{-1} was used as a supplemental fertilizer while 15+60 kg N ha^{-1} was used as basal and supplemental fertilizer in wheat. Calcium super phosphate (150 kg P_2O_5 ha^{-1}) to NPK, NP and PK treatments, potassium sulfate (150 kg K_2O ha^{-1}) to NPK, NK and PK treatments, and organic manure (adding 1164 kg C ha^{-1} and 150 kg N ha^{-1} to OM treatment, and 582 kg C ha^{-1} and 75 kg N ha^{-1} to HOM treatment) were applied as the basal amendments.

For making organic manure, the organic sources were wheat straw, oil cake, and cottonseed cake used with mass ratio of 100:40:45, respectively. These materials were machine ground, mixed with water, and composted for two months. The organic manure had 7.81 C:N ratio containing 422 g C kg^{-1}, 54 g N kg^{-1}, 18 g P_2O_5 kg^{-1}, and 24 g K_2O kg^{-1} (Meng *et al.*, 2005; Ding *et al.*, 2007). After applying the basal fertilizers and manure to the soil surface, the soil was immediately plowed before plantation of maize (early June) and wheat (early October). Supplementing fertilizer doses were added in the wheat and maize crops in late February and late July, respectively, followed by irrigation. Noticeable weeds were removed manually.

***Soil sampling and analysis*:** During September 2012, ten soil samples were drawn from every treatment plot with auger (having 2.5 cm diameter and 20 cm blades length). Soil samples were thoroughly mixed to get a composite sample. Moist samples were gently crushed and screened with 8 mm sieve. From the sieved soil, plant residues were carefully removed with forceps. After thorough mixing, soil water content was measured by drying a sub-sample at 105°C. Another sub-sample was air-dried to determine other soil properties. Leftover moist samples were subjected to wet-sieving for aggregates fractionation. Further, wet oxidation and Kjeldahl procedures were employed to measure SOC and total N (TN), respectively (Nelson and Sommers, 1982; McGill and Figueiredo, 1993).

***Soil aggregates fractionation*:** For the determination of soil aggregates size fractions, wet-sieving protocol was adopted by placing 100 g of moist soil sample on top-surface of a 250 µm sieve and immersed in distilled water (>1 mm macro-aggregates) for 5 min at ambient temperature. Sieve was manually moved 3 cm up and down for 50 times within 2 min. Leftover portion on 250 µm sieve was collected in aluminum pan. For particles ＜250 µm in size, soil was passed from 53 µm sieve, and repeated the sieving step for 50 times within 2 min. For getting soil fraction with a particle size of ＜53 µm, supernatants of the second sieving step were shifted to 250 mL centrifuge tubes operated at 5000 × *g* for 30 min at 4°C. Pellets were re-suspended in distilled water, and centrifuged three times as before. Thus, macro-aggregates (>250 µm), micro-aggregates (53−250 µm) and silt + clay fraction (<53 µm) from the test soil were separated.

***Amino acids assay*:** Soil amino acids were measured by gas chromatography as described by Zhang and Amelung (1996). Briefly, 200 mg of air-dried and screened (<0.25 mm) soil was hydrolyzed by treating with 20 mL of 6 M HCl for 12 h at 105°C in oven. Then cooled at room temperature and added 200 µL of 1st internal standard of norvaline. Hydrolysate from treated sample was filtered using glass fiber filter (GF 6), and dried by rotary evaporator at 45°C. Final solution was purified in 25 mL polypropylene column; added 3 g of DOWEX 50WX8 cation exchange (CE) resin 200 mesh in polypropylene sample preparation column. Pre-rinsed the CE resin with 25 mL 2 M HCl step by step, then washed with 25 mL of 2 M NaOH, and at the end washed with DI water to achieve neutral pH.

Dried amino acids in evaporation flasks were dissolved with 4 mL of 0.05 M HCl and transferred to CE resin column. Flasks were re-washed with 4 mL of 0.01 M HCl, and supernatant solution was inserted in to same resin column. This column was washed with 25 mL of 0.1 M oxalic acid to remove metals. Finally, it was washed with 5 mL of 0.01 M HCl and 5 mL DI water, then added 25 mL of 2.5 M NH_4OH and solution was collected in rotary evaporation flasks and dried at 45°C. Sample was then solubilized with 4 mL of 0.1 M HCl, transferred to centrifuge tube operated for 15 min at 4200 × *g* and poured into 5 mL vial. After centrifugation, along with 175 µL of 2nd internal standard (having ca. 70 µg d-methionine), sample solution was completely frozen at –18°C for >3 h, and then freeze-dried. Then, added freshly prepared isopropanol (1:2.5, v/v) at 0°C in a methanol bath, and 400 µL of 4 M HCl in the freeze-dried sample. Vial was firmly closed with a Teflon-laminated septum and heated for 30 min at 110°C, cooled to ambient temperature, and supernatant solution was transferred to smaller vial (2 mL). Then, 130 µL each of dichloromethane and pentafluoropropionic anhydride were mixed in processed sample, and the vial was heated again for 10 min at 110°C. After cooling to ambient temperature, the solution was dried under N_2 stream. Dried amino acids were dissolved in 150 µL dichloromethane, shacked for 30 min, and supernatant solution was shifted to GC auto-sampler vial.

Amino acids were assayed on GC-MS instrument (QP 2010 PLUS; Shimadzu, Japan) coupled with flame-ionization detector. A 30 m capillary column (25 m prolonged by 5 m, 0.25 mm ID, 0.12 µm film thickness) coated with Chirasil-L-Val (Chrompack, The Netherlands) was used to separate amino acid derivatives with a split ratio of 40:1. Helium was run as carrier gas, and system was driven at constant column and make-up-flow conditions (25 mL min^{-1}). Temperature was programmed as described by Zhang and Amelung (1996).

***Data processing and statistical analysis*:** Statistical differences among fertilizer treatment means data were worked out through one-way analysis of variance followed by least significant difference test using SPSS v-24 (IBM SPSS Corp., USA) as described by Sokal and Rohlf (1997). Linear regression model was fitted to find out the relationship between amino acids and soil properties. Figures were drawn through Origin Pro 8.5 software (Origin Lab Corp., Northampton, USA).

RESULTS

***Soil properties*:** Various combinations of organic and mineral N, P and K fertilizers input had their residual impact on biochemical, biological and physico-chemical attributes of arable field soil (Table 1). The SOC contents were at the highest under the OM (12.20 g C kg^{-1}) followed by HOM (9.28 g C kg^{-1}) treatment with significant ($P \leq 0.05$) difference, and the lowest one in control. Variation in SOC contents under different treatments depended on the input of organic and mineral nutrient sources affecting the processes of mineralization and nitrification ultimately C and N balance in soil. Next highest SOC contents were in NPK- and NP-treated soil; however, NK treated soil rendered non significant difference with SOC content in control. The C:N

Table 1. **Effect of different organic and mineral fertilizer combinations on various soil properties after 23 years of treatments.**

Treatments	SOC (g C kg^{-1})	C:N	Total N (g N kg^{-1})	pH	Mass proportion of aggregates (%) M	m	SC	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})
OM	12.20a	7.82	1.56a	8.01b	30.8a	55.1c	14.1c	18.26a	0.93
HOM	9.28b	8.43	1.10b	8.19b	17.7b	62.9b	19.4b	14.66b	ND
NPK	7.00c	8.75	0.80cd	8.17b	8.8d	70.1a	21.1b	10.27c	0.81
NP	6.64c	8.73	0.76cd	8.01b	10.1d	68.5a	21.4b	12.41c	0.69
PK	5.80d	9.36	0.62de	8.35a	14.8c	63.1b	22.1b	6.96d	ND
NK	5.22e	5.93	0.88c	8.58a	9.9d	61.9b	28.2a	6.78d	ND
Control	4.42e	9.4	0.47e	8.61a	8.8d	71.5a	19.7b	5.04d	0.90

Treatments comparison data in each individual column bearing dissimilar letter (s) have statistically significant difference among them at $P \leq 0.05$. Abbreviations express soil aggregate categories as: M, macro-aggregates; m, micro-aggregates; SC, free silt+clay fraction; ND, not detectable amount. Treatments detail is as: whole N from organic manure (OM); half N from organic manure + half N from inorganic fertilizer (HOM); mineral NPK fertilizers (NPK), NP, NK, PK, and no fertilizer (control).

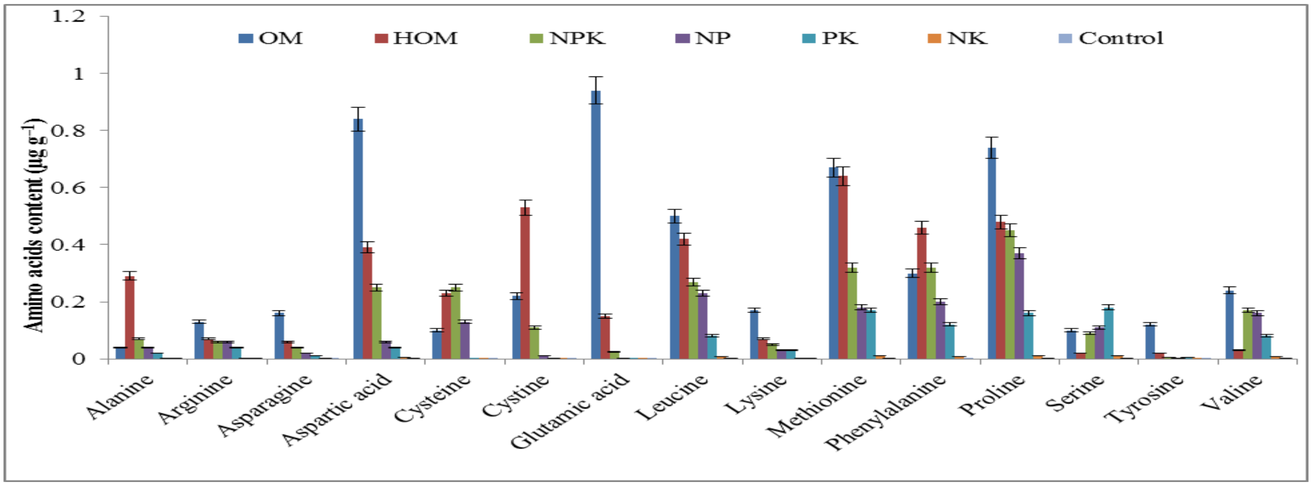

Figure 1. **Effect of different organic and mineral fertilizer combinations on amino acids content.** Treatments detail is as: whole N from organic manure (OM); half N from organic manure + half N from inorganic fertilizer (HOM); mineral NPK fertilizers (NPK), NP, NK, PK, and no fertilizer (control).

ratio of soil among all treatments had no statistical difference, although NK (5.93) and OM (7.82) had the lowest values, and control showed the largest C:N value. Contents of total N in soil were statistically ($P \leq 0.05$) higher with the OM (1.56 g N kg^{-1}) and HOM (1.10 g N kg^{-1}) treatments by comparing with NPK (0.80 g N kg^{-1}), NP and NK which differed non significantly among themselves, all being superior to control (0.47 g N kg^{-1}) and PK treatment. The pH values were slightly lower than that of original soil (8.65), being highest in control (8.61) followed by NK (8.58) and PK (8.35) differing non significantly. The lowest pH was under OM and NP treatments with a value of 8.01.

Mass proportions of macro-aggregates (M) were significantly ($P \leq 0.05$) enhanced with manure amendments as 30.8% under OM and 17.7% through HOM, while the lowest percentage (8.8%) was obtained from NPK and control. However, the proportions of micro-aggregates (m) were reduced in the OM and HOM treatments, showing results just the opposite to that of macro-aggregates. Thus treatments of control (71.5%) and inorganic fertilizer NPK (70.1%) showed the highest mass proportion of micro-aggregates. Free silt + clay (SC) fraction reflected significant reduction with OM (14.1%) and HOM (19.4%) amendments, while the highest percentage was under fertilizer NK (28.2%) followed by PK (22.1%) treatment. Control (19.7%) had non significant difference with HOM and other fertilizer treatments. Nitrogen contents were greater in NO_3^-–N form as compared to NH_4^+ component. The highest contents of NO_3^-–N were detected in organic manure treatments, viz., 18.26 and 14.66 mg kg^{-1} with OM and HOM, respectively, followed by NP and NPK fertilizers, and the lowest (5.04 mg kg^{-1}) in control plot soil. Contents of NH_4^+–N were inconsistent with the nature of treatments as they were not in detectable amount under HOM as well as fertilizer combinations of PK and NK. The NH_4^+–N content was also highest under OM treatment (0.93 mg kg^{-1}) followed by control plot (0.90 mg kg^{-1}), while NPK and NP

Table 2. Correlation of amino acids with SOC, macro-aggregates, micro-aggregates, and silt+clay fraction.

Regression Attributes	Arginine	Lysine	Proline	Leucine	Serine	Methionine	Phenylalanine	Aspartic acid	Asparagine	Tyrosine	Alanine	Valine	Cysteine	Cystine	Glutamic acid
Correlation between amino acids and SOC															
R^2 value	0.9524	0.9753	0.9355	0.9517	0.1565	0.9479	0.7532	0.9749	0.9705	0.8927	0.4606	0.6682	0.4829	0.6842	0.8893
Prob.	0.0009	0.0002	0.0020	0.0010	0.7376	0.0012	0.0506	0.0002	0.0003	0.0068	0.2983	0.1009	0.2724	0.0900	0.0074
Correlation between amino acids and macro-aggregates															
R^2 value	0.8355	0.9211	0.7382	0.7491	0.2263	0.7987	0.4654	0.9087	0.9267	0.9525	0.2381	0.5421	0.0857	0.5030	0.9459
Prob.	0.0193	0.0032	0.0582	0.0526	0.6256	0.0313	0.2926	0.0046	0.0027	0.0009	0.6071	0.2087	0.8550	0.2499	0.0013
Correlation between amino acids and micro-aggregates															
R^2 value	-0.5992	-0.7010	-0.4834	-0.5071	-0.1906	-0.5874	-0.2537	-0.6917	-0.7106	-0.7772	-0.1421	-0.3197	0.1182	-0.3686	-0.7703
Prob.	0.1551	0.0793	0.2717	0.2455	0.6822	0.1656	0.5831	0.0851	0.0735	0.0398	0.7611	0.4845	0.8008	0.4158	0.0427
Correlation between amino acids and silt+clay fraction															
R^2 value	-0.7747	-0.7991	-0.7474	-0.7360	-0.1711	-0.7208	-0.5410	-0.7881	-0.7967	-0.7549	-0.2600	-0.5971	-0.3248	-0.4557	-0.7518
Prob.	0.0408	0.0311	0.0535	0.0593	0.7137	0.0676	0.2099	0.0353	0.0320	0.0498	0.5734	0.1569	0.4772	0.3041	0.0513

treated soils had 0.81 and 0.69 mg kg^{-1} of NH_4^+–N content, respectively. These results did not give any clear reflection of the treatments effect on NH_4^+–N content in the soil.

***Amino acids composition*:** Within all the soil samples from the experiment treatments, only 15 out of 22 amino acids could be detected (Fig. 1). Order of total amino acid contents (µg g^{-1}) in various treatments was: OM (5.34) > HOM (3.94) > NPK (2.54) > NP (1.73) > PK (0.92) > NK (0.08) > control (0.006). Among these amino acids, under OM treatment the most abundant were glutamic acid, aspartic acid, proline, methionine and leucine with their corresponding contents as 0.94, 0.84, 0.74, 0.67 and 0.50 µg g^{-1}. While the highest content in the HOM treatment were found to be methionine (0.64 µg g^{-1}) followed by proline (0.48 µg g^{-1}) and phenylalanine (0.46 µg g^{-1}). In contrast, the proline content was highest in the inorganic fertilization treatments. By comparing with control, fertilization also enhanced the contents of other amino acids including methionine, phenylalanine, leucine and valine to some extent. Aspartic acid and cysteine having same value (0.25 µg g^{-1}) were found also in significant amount under NPK treatment.

Glutamic acid was lesser in the soils treated with NPK (0.024 µg g^{-1}), NP (0.0033 µg g^{-1}), and PK (0.00037 µg g^{-1}) if compared to OM (0.94 µg g^{-1}) and HOM (0.15 µg g^{-1}). The concentrations of leucine, methionine, phenylalanine and proline were mostly higher as compared to other amino acids in all the treatments except that under OM where glutamic acid and aspartic acid were greater. Soils amended with OM and HOM rendered a statistical increase ($p \leq 0.05$) over control in the contents of majority amino acids than yielded by two and three combinations of N, P and K mineral fertilizers (Fig. 1). Under NP, PK and NK fertilizer treatments, amount of observed 15 amino acids was very low; whereas, the amount of amino acids was greater in organically treated soils if compared with NPK fertilized soil.

***Correlation between amino acids and soil properties*:** Regression analysis of amino acids contents indicated that arginine, proline, and methionine were significantly correlated ($R^2 = 0.92$, $P \leq 0.01$) with SOC contents (Table 2). Similarly, lysine, leucine, phenyl-alanine aspartic acid and valine were correlated significantly ($R^2 = 0.6605$, $P \leq 0.05$) with SOC contents. Serine, alanine, cystine and cysteine had smaller individual R^2 values and were correlated non significantly with SOC contents in soil. Remaining three amino acids (asparagine, tyrosine and glutamic acid) out of total 15 had very high R^2 values (0.89-0.97) and were significantly correlated ($P \leq 0.05$) with SOC content in soil. A significant association was also observed for mass proportion of macro-aggregates with valine, lysine, proline, methionine, aspartic acid and tyrosine (Table 2). However, mass proportion of micro-aggregates showed a significant relationship with serine. Remaining majority of amino acids had poor or negative correlation with micro-aggregates. Correlation between amino acids and silt+clay fraction was positively significant for proline but significantly negative ($P \leq 0.05$) for arginine, lycine, leucine, aspartic acid, asparagine, tyrosine and glutamic acid.

DISCUSSION

***Nutrient management vs. soil characteristics*:** Increased use of organic matter is essential for enhancing the crop productivity and ability of cropland soil to perform as C-sink (Wu *et al.*, 2018). Findings of our long-term (23 years) field research highlight the importance of optimized integration of organic manure and NPK mineral fertilizers in C sequestration for higher farmland productivity. The SOC contents were statistically higher with the use of full and half rates of compost + ½NPK fertilizers (OM and HOM, respectively) as compared with the sole NPK- and NP-treated soil (Table 1). Because compost used in this experiment contained 422 g C kg^{-1}, 54 g N kg^{-1}, 18 g P_2O_5 kg^{-1}, and 24 g K_2O kg^{-1}, therefore, SOC contents under various treatments depended on the input amount of organic and mineral nutrient sources. These sources influence the processes of mineralization, nitrification and ultimately the balance of C and N in soil. Greater bioavailability or addition of N renders positive influence on the stabilization of fresh microbial residues by inhibiting mineralization and transformation of original organic residues-N into microbial residues over long time (Zhang *et al.,* 2015). Compost directly contributed the SOC and nutrients which sufficiently fulfilled the microbial food needs whose necromass was later conserved as more stable SOM (Kögel-Knabner, 2017). Further, SOM was enhanced by root biomass, leaf residues, rhizo-deposition components of both crops, and directly applied organic manure (Bhattacharyya *et al.,* 2010).

Application of NPK+FYM rendered greater C accumulation and sequestration in soil, improved aggregation, and gave 26% higher C management index than with NPK alone (Ghosh *et al.,* 2018). Among the di-nutrient fertilizer combinations, NP performed slightly lower than NPK without statistical difference for SOC content; however, it was significantly superior to PK and NK. These results indicate that among NPK nutrients, P is more relevant towards SOC acquisition, and K had the least role in this aspect. Phosphorus is component of nucleotides and energy compounds in both plants and microbial cells (Stevenson, 1994). Thus its availability in greater amount leads to higher plant and microbial biomass production, whose residues highly contribute to SOC. Zhao *et al.* (2017) reported a similar response of N, P and K use with and without compost addition for improving SOC content after 18 years of continuous field experiment. Slightly lower C:N and pH along with greater TN content under OM and HOM treatments as compared to sole fertilizer treated soils further signify the importance of appropriate nutrient supply mainly N and P along with organic C input.

Begum *et al.* (2017) employed DDC simulation model to forecast SOC sequestration and monoculture wheat yield under various treatments of fertilization. For 170 years, it simulated the highest rise of SOC in the FYM plot (3 times greater than in control). Higher sensitivity (4–7%) of predicted SOC changes was noted for variations in external C input. Extent of SOC stabilization depends on several factors, viz., weather conditions, soil management and properties, nutrients input (fertilization), and straw return (Han *et al.,* 2018). The most limiting nutrient for enhancing the soil quality was P, followed by N and K; thus balanced fertilization including organic amendments is crucial in this regard (Zhao *et al.,* 2013). Meta-analysis of data from 84 long-term experiments by Zhao *et al.* (2017) revealed that C storage in soils using NPK, N, P, and K was correspondingly 10, 5, 5 and 2% greater than in unfertilized fields. Synergistic use of manure (10–15 Mg ha^{-1} yr^{-1}) along with NPK sequestered annually 50.7–900 kg SOC ha^{-1} over 28–33 years (Manna *et al.,* 2017). Proportion of organic materials input converted to SOC could be around 19% of the original C; the remaining is either used by soil biota or lost as CO_2 gas. Further, the gain in SOC over sole NPK was 17.18 Mg C ha^{-1} with the addition of FYM (10 Mg ha^{-1}) plus NPK fertilizers over a period of 30 years (Bhattacharyya *et al.,* 2010).

Mass proportion of macro-aggregates was significantly enhanced with OM and HOM treatments, while the lowest one was obtained from NPK and control. Contrastingly, the micro-aggregates and free silt+clay proportions decreased with the OM and HOM treatments. Appropriate use of NPK, sole or integrated with manure, improves OC sequestration by uniformly enhancing the aggregate-associated OC and ensuring the same level of protection for OC in both macro- and micro-aggregates (Xie *et al.,* 2017). Proportion of micro-aggregates (55.1-71.5%) was far greater than that of macro-aggregates (8.8-30.8%). Bhattacharyya *et al.* (2010) reported that SOC contents with huge size of aggregates were higher than that of small size aggregates, and the 0.1-0.25-mm size part preserved maximum SOC stock in sandy loam soil. Further, the proportion (%) of macro-aggregates was improved, and micro-aggregates declined with addition of FYM and NPK if compared to mineral fertilizer treatments (NP, NK and NPK). Stability of soil aggregates significantly enhances with the application of FYM alone as compared to integration with mineral fertilizers (Diallo-Diagne *et al.,* 2016). With respect to physical properties, application of mature compost rather than young one favours larger and stable aggregates formation (Pernes-Debuyser and Tessier, 2004). Larger proportion of aggregates with FYM application has been attributed to more and fresh organic residues, which enhances C bioavailability and microbial activity in the soil (Mikha and Rice, 2004). Use of chemical fertilizers (N, NP and NPK) has been found to reduce the macro-aggregates (Manna *et al.,* 2006). Micro-aggregates usually accumulate within the macro-aggregates through binding agents like plant roots, fungal hyphae and microbially produced extracellular polymeric substances (EPS) and their biomolecules as polysaccharides and proteins (Six and Paustian, 2014; Totsche *et al.,* 2018). In micro-aggregates entrapped within macro-aggregates, the relatively higher C content in OM-amended soil exhibits potential in bringing higher C stability via integration of inorganic and organic manures and crop remains.

Nitrate-N contents of the soil were greater than NH_4^+–N, with the highest contents of NO_3^-–N under OM and HOM followed by NP and NPK fertilizer treatments. Organic manures stimulate biological N_2 fixation by providing a direct source of OC required by diazotrophs and other soil organisms, and also the indirect C source through enhanced plant growth and their residues (Bitew and Alemayehu, 2017). These phenomena also contributed considerably to nitrogen in the OM included treatments over sole NPK. Mineral fertilizers especially NPK or NP together increased the content of nitrate–N in soil over the control plots, whereas, PK and NK combinations did not differ significantly with control. Thus, integrated nutrient management with residue recycling has been recommended recently for enhancing the system's productivity, C and N sequestration and microbial biomass C and N enhancement in soil (Yadav *et al.,* 2017).

***Impact of nutrient management on soil amino acids*:** During the course of SOM transformation, amino acids come towards the last product in the labile as well as reserve pool of SOM being considered for C and N sequestration in soil (Zanella *et al.,* 2018). Under this long-term study, amount of total amino acids was at its maximum (5.34 µg g^{-1}) with the sole use of OM full dose being significantly higher than that with HOM (half rate of both OM and NPK fertilizers) and then followed by rest of the N, P and K fertilizer combinations (Fig. 1). These results signify the variable role of organic manure as well as inorganic N, P and K fertilizers for SOM stabilization in soil. Among the individual amino acids, proline got higher value under NPK and NP but not for OM and HOM treatments (Figure 1). Table 2 indicates greater positive correlation of proline with SOC and macro-aggregates, but negative correlation with micro-aggregates and silt+clay fraction. Proline is a hydrophobic amino acid (low solubility in water) and is located in the center of protein molecules (less prone to biodegradation), therefore, it could be having relatively more residual properties than other amino acids. Secondly, ratio of single amino acid / total amino acids increases progressively with decomposition stage (Werdin-Pfisterer *et al.,* 2009). As the application of quickly soluble / available mineral NPK fertilizers enhances the organic matter decomposition in soil more than that with sole OM treatment, so it increased the proline contents in soil.

From the total N in soil, about 96% occurs in organic-N forms comprising mostly dead OM and humic substances (Schlesinger, 1991; Stevenson, 1994); and the rest 4% of earthly organic N found in living biomass (Stankiewicz and Van-Bergen, 1998) is dominated (94–99% N) by plants, and only 1–6% is the component of soil animals and microorganisms (Stevenson, 1994). Hence, organic-N (mainly amino acids) present in the SOM contributes largely to the N reservoir on earth's surface. The most emphasized total pool of OC in soil has a mean residence time (MRT) of merely 26-40 years (Schlesinger, 1991). It signifies that N, mostly in the organic form (likely as amino acids) is conserved in soil for long times. Similarly, phosphorus being the major component of nucleotides and energy compounds used or acquired by soil microorganisms during OM transformations and turnover; it contributes significantly in C and N sequestration. Organically bound P comprises mostly above 50% of the total P (100-900 mg kg^{-1}) in soil (Stevenson, 1994), sometimes with a low range of 15–20 or very high as 80–90% (Stevenson and Cole, 1999) with MRT of 350-2000 years (Paul and Clark, 1996). All these background facts about N and P indicate their great role in terrestrial geochemical cycles including that of C, N, P and S, which if operated harmoniously, could address the environmental issues like pollution, global warming and GHG emissions to a great extent.

Higher content of amino acids recorded in OM-treated soil than from any of the HOM, N, P and K combinations, is attributed mainly to the bioavailability of required nutrient elements along with OC for the soil microorganisms engaged in SOM transformation. Total amino acid contents in soils were correlated with SOC positively and significantly, the same has been reported previously by Werdin-Pfisterer *et al.* (2009). Advanced stages of organic matter or litter decomposition correspondingly increased the content of glutamic acid in soil. Rovira *et al.* (2008) discovered ordinary mimic of decay behavior (progressive increase or decrease) for some amino acids. Ratio of single amino acid / total amino acids for Gly, Ala and Thr increased progressively with decomposition stage. Such distinct behavior of individual amino acids in soils is due to differences in adsorption pattern of these amino acids, which basically depends on the presence and content of SOC in soils (Werdin-Pfisterer *et al.,* 2009). While characterizing the distribution of soil amino compounds in 11 major Chinese croplands, Cao *et al*. (2018) demonstrated that free amino acid contents ranging from 0.26 to 1.03 mg N kg^{-1} were about seven times lesser than the soil-adsorbed amino acids. In spite of great variations in soil characteristics and environment, seven amino acids with similar composition dominated in all soils, viz., alanine, glycine, glutamic acid, aspartic acid, serine, threonine, and isoleucine, suggesting their similar biochemical dynamics and constituents.

***Conclusion*:** This study provides a new vision about sequestration of organic carbon along with nitrogen (in the form of amino acids) in crop growing soils, and the effects of organic as well as mineral fertilizers on soil amino acids concentration. Results suggested significant changes in amino acids concentration after addition of fertilizer. However, these variations differed with the type of fertilizer, viz., OM, HOM, and NPK. Decomposition of higher C:N organic amendment (straw) is the primary process that supplies amino acids and increases their net contents. After 23-years of soil amendment with OM, HOM and NPK fertilizer, the increased SOC content under manure treatments accelerated the formation of macro-aggregates, but percentage of micro-aggregates was greater with mineral NPK fertilization and without any amendment. Thus, combining the soil aggregation with amino acid biomarkers analysis could elucidate that how and to what extent the microbial-derived residues in response to differential manure / fertilizer (N, P and K) inputs influence the C-sequestration in these soil microhabitats.

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