

LONG-TERM FERTILIZATION EFFECTS ON ORGANIC CARBON, AGGREGATES, AND L- AND D-AMINO ACIDS IN SOILS UNDER WHEAT AND MAIZE CROPS

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Abstract

Soil organic carbon (SOC) plays a key role in maintaining soil quality and improving soil physical, chemical, and biological properties. Long-term organic and inorganic fertilization effects on SOC, soil aggregates, and L- and D-amino acids under wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) crops were studied at Fengqiu State Key Agroecological Station, Fengqiu County, China. Field studies were initiated in September 1989 and continued for 23 years until 2012. Compost manure and NPK fertilizers alone and in different combinations were applied to wheat and maize crops. Wheat and maize as test crops were grown during winter and summer cropping seasons, respectively. The field experiment included seven treatments i.e., a) compost (CM), b) combination of half compost plus half inorganic fertilizer N (HCM), c) inorganic NPK (nitrogen-phosphorus-potash) fertilizer, d) inorganic NP fertilizer, e) inorganic PK fertilizer, f) inorganic NK fertilizer, and g) no fertilizer (control) were applied in a randomized complete block design. The L- and D-amino acids concentrations were significantly enhanced in CM- and HCM-treated soils. Amino acids showed a positive response in organically fertilized soil. The L-aspartic, L-glutamic, and D-aspartic acids were significantly ($p \leq 0.01$) and positively associated with SOC content. Similarly, SOC content showed a significant positive correlation with L-alanine, L-lysine, L-phenyl-alanine, L-glutamine, and D-tryptophan acids. The D-tryptophan and D-aspartic acids showed a significant positive correlation with total nitrogen. Organic fertilizer alone and in combination with NPK fertilizers increased the amino acids which ultimately enhanced soil fertility by increasing SOC.

Key words: Long-term fertilization, Organic manure and inorganic fertilizers, Soil properties, Soil organic carbon, L- and D-amino acids, *Triticum aestivum* L., *Zea mays* L.

Introduction

A plant's ability to take up amino acids is common, however, the ecological and physiological implications of this ability are not fully understood. Soil amino acids are mainly derived from soil microbes, animal and plant residues, and their metabolites (Friedel & Scheller, 2002; Zhang *et al.*, 2017; Bibi & Ding, 2018). Amino acids mainly exist in the form of proteins or polypeptides, which are tightly bound and protected by humus and soil inorganic components, such as iron and aluminum hydroxide (Jones *et al.*, 2005; He *et al.*, 2006; Kolukisaoglu, 2020). Following the number of amino and carboxyl groups, amino acids are classified as a) neutral amino acids, such as alanine, valine, glycine, isoleucine, leucine, tyrosine, proline, serine, and phenylalanine; b) basic amino acids, namely, histidine, arginine, and lysine; and c) acidic amino acids, including aspartic and glutamic acids.

In the soil system, amino acids, which are important components of soil nitrogen compounds, account for 10% to 30% of the total soil carbon and have important implications for soil carbon and nitrogen cycling processes (Amelung & Zhang, 2001; Bibi *et al.*, 2018; Kolukisaoglu, 2020). The mechanism is necessary to understand the conversion of carbon into various soil amino acids. In the present study, gas chromatography-mass spectrometry (GC-MS) was used to quantify the

enrichment ratios of different L- and D-amino acids (Reis *et al.*, 2020). The reserves of SOC (1500 PG) are approximately 2.4 times those of terrestrial biomass carbon (620 PG); the content and dynamic changes of SOC directly affect soil fertility and crop yields, and the loss of SOC leads to soil degradation, which is accompanied by reductions in crop yield (Lal, 1999; Manlay *et al.*, 2007, Bibi *et al.*, 2010).

An understanding of the transformation and accumulation of farmland SOC is not only an important basis for the sustainable use of land resources but also the synthesis of the relationship between soil carbon cycling and global climate change (Tian *et al.*, 2010; Zhao *et al.*, 2019, 2020). The prolonged application of NPK fertilizers alone or in combination with organic manures has positive and variable effects on total OC (TOC) and significantly increases SOC and total soil porosity while decreasing soil bulk density in surface soil (Chaudhary *et al.*, 2017; Hao *et al.*, 2017; Bibi *et al.*, 2019). Compared with the control treatment, fertilization considerably increases SOC, macroaggregates, and the silt + clay (SC) fraction but not micro aggregates, whereas compost application substantially reduces the specific activities per unit organic C in soil and aggregate sizes (Yu *et al.*, 2012a). Soil temperature, humidity, aeration, pH value, and other physical and chemical properties change organic carbon (OC) transformation by affecting soil microorganisms (Robinson *et al.*, 1994).

Fertilization mainly affects SOC transformation and accumulation by a) improving crop biomass yield, thus increasing residue and root contents in the soil and changing microbial populations and structures, and b) affecting SOC transformation (Fierer *et al.*, 2001). The long-term application of fertilizers, especially organic compost, can increase the degree of soil reunion and OC content, as well as promote the aggregation of organic matter with soil particles of different sizes (Aoyama *et al.*, 1999; Bibi *et al.*, 2018, 2019; Zhanhui *et al.*, 2018). Organic manure amendment significantly elevates OC concentrations in aggregates and aggregate sub-fractions, including intra-particulate organic matter and SC sub-fractions (Zhao *et al.*, 2019, 2020). Increasing nitrogen application considerably increases amino acid content in the soil (Zhang *et al.*, 2017).

The use of organic manure mainly increases the OC content of large soil aggregates (>250 μm), whereas the application of inorganic fertilizers increase the OC content of silty and clay particles (Meng *et al.*, 2005; Yu *et al.*, 2012b). The increment in SOC contents may differ because of the various mechanisms of soil microorganisms. The turnover rate of a series of organic compounds is affected by the differences in the stability of their molecular structure, the characteristics of different OC sources, and the variations in the degree of their protection in the soil. Carbohydrates (10–20%) are important components of the total SOC (Guo & Giford, 2002), belong to the active OC pool, and are significantly correlated with microbial activity because of the main energy and carbon sources of soil microorganisms (Hu *et al.*, 1997). The combination of carbon and water can also improve the formation and soil matrix position of soil aggregates, and the combination of soil aggregates with mineral particles can also affect SOC degradation (Schulten & Leinweber, 2000; Zhanhui *et al.*, 2018).

The microbiological, chemical and physical processes that occur during SOC transformation can be partially attributed to the kinetic processes of soil carbon compounds (Amelung & Zhang, 2001; Kolukisaoglu, 2020). In soil under different long-term organic and inorganic fertilization regimes, microbial transformation increases the accumulation of OC and the entry of microbial communities into aggregates with different particle sizes (Bibi & Ding, 2018). The classification of aggregate structure and definition of the relationship of aggregates with OC accumulation provides the basis for the formulation of fertilization measures that favor increasing OC contents and improving soil strength in alluvial plain soils in China.

The present study aimed to determine the effects of long-term organic and inorganic fertilization for 23 years (1989 to 2012) on a) the concentration of amino acids, b) the differences between L- and D-amino acid concentrations and their correlation with soil properties, and c) the differences between organic and inorganic nitrogen concentrations under wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) cropping systems.

Materials and Methods

Study site and procedure: The effects of long-term organic and inorganic fertilization on soil L- and D-amino acid profiles and soil properties under wheat and maize cropping were studied at Fengqiu State Key Agro-ecological Station, Fengqiu County, China (35°00'N, 114°40'E). Long-term field studies were initiated in September 1989 and continued for 23 years until September 2012. Compost manure and NPK fertilizers were applied alone and in different combinations to wheat and maize crops. The wheat and maize crops were grown during the winter and summer cropping seasons, respectively. Soil analysis was performed in September 2012 before the commencement of the present research. The soil of the study site had a sandy loam texture with sand, silt, and clay percentages of 52%, 33%, and 15%, respectively; pH of 8.65; and OC and total nitrogen (TN) contents of 4.48 and 0.43 g kg⁻¹, respectively (Zhang *et al.*, 2015).

The field experiment included seven treatments i.e., a) compost (CM), b) combination of half compost plus half inorganic fertilizer N (HCM), c) inorganic NPK (nitrogen-phosphorus-potash) fertilizer, d) inorganic NP fertilizer, e) inorganic PK fertilizer, f) inorganic NK fertilizer, and no fertilizer (control). All these treatments were arranged in a randomized complete block design (RCBD) with four replications (9.5 m × 5 m per plot). In the NPK, NP, and NK treatment plots, nitrogen was applied in the form of urea at the rate of 150 kg N ha⁻¹ in two splits, i.e., 60:90 kg N ha⁻¹ as basal and supplemental doses to maize crops and 90:60 kg N ha⁻¹ as basal and supplemental doses to wheat crops.

Under HOM treatment, 75 kg N ha⁻¹ urea was used as a supplemental fertilizer for maize crops, whereas 15 and 60 kg N ha⁻¹ were used as basal and supplemental fertilizers, respectively, for wheat. P₂O₅ (150 kg ha⁻¹ for NPK, NP, and PK), K₂O (150 kg ha⁻¹ for NPK, NK, and PK), and organic manure (1164 kg ha⁻¹ C and 150 kg ha⁻¹ N for OM treatment and 582 kg ha⁻¹ C and 75 kg ha⁻¹ N for HOM treatment) were applied as the basal amendment. Basal manures and fertilizers were applied to the soil surface, and the soil was plowed immediately before the cultivation of maize in early June and wheat in early October. Supplemental fertilizers were also applied to the soil surface for wheat and maize crops in late February and late July, respectively, following rainfall or irrigation. The same field management practices were applied for all the treatment groups. Noticeable weeds were removed by hand. At harvest, the wheat and maize crop plants, except for their stubbles and roots, were removed completely from the field. After each crop, the fields were tilled with a conventional cultivator for land preparation and to grow the next crop.

Traits measurement

Soil sampling and analysis: In September 2012, 10 soil samples were collected up to a depth of 20 cm from each plot by using 2.5 cm-diameter augers. The soil samples were thoroughly mixed to form one composite sample. Moist soil samples were gently broken apart along natural breakpoints and passed through an 8 mm sieve. Plant and organic debris in the sieved soil were carefully removed

with forceps. Soil moisture was measured by drying a sub-sample at 105°C after thorough mixing. Another sub-sample was air-dried for the analysis of soil properties. The remaining moist soil was wet-sieved for the analysis of water-stable aggregates. The wet oxidation and Kjeldahl methods were used to measure SOC and TN, respectively (Carter, 1993).

Soil fractionation: The aggregates of moist soils were separated by using the wet-sieving protocol (Elliott, 1986). Briefly, 100 g (on an oven-dried basis) of the moist soil sample was placed on top of a 250 µm sieve and submerged in deionized water (no >1 mm macroaggregates) for 5 min at room temperature. The sieve was manually moved up and down by 3 cm. This process was repeated 50 times within 2 min. The portion left behind on the 250 µm sieve was collected into an aluminum pan. The sample was passed through a 53 µm sieve, and the sieving procedure was repeated to collect soil particles that were <250 µm in size. The supernatants of the second sieving step were transferred into 250 mL centrifuge tubes and centrifuged at 5000 × *g* for 30 min at 4°C to obtain the soil fraction with a particle size of <53 µm. The pellets were re-suspended in deionized water and re-centrifuged three times under the same conditions. The macroaggregates (>250 µm), micro aggregates (53–250 µm), and the SC fraction (<53 µm) of the test soils were thus separated. The recovery rates of the different fractions after wet-sieving were 96.0 to 99.0%.

Amino acids assay: Soil amino acids were measured through gas chromatography (GC) as described by Zhang & Amelung (1996). Briefly, 200–250 mg of the air-dried and screened (<0.25 mm) soil sample for L- and D-amino acids (1 g of soil sample was used for free amino acid analysis) was weighed and then hydrolyzed with 20 mL of 6 M HCl for 12 h at 105°C in the oven. The sample was then cooled at room temperature and mixed with 200 µL of the first internal standard of norvaline. The hydrolysates from the treated samples were filtered by using glass fiber filters (GF 6) and dried with a rotary evaporator at 45°C. The final solution was purified in a 25 mL polypropylene column and then mixed with 3 g of 100–200 mesh Dowex 50 W X8 cation exchange (CE) resin in a polypropylene sample preparation column. The CE resin was pre-rinsed with 25 mL of 2 M HCl in a stepwise, not collective, manner; washed with 25 mL of 2 M NaOH; and finally washed with deionized water to a neutral pH.

Dried amino acids were dissolved with 4 mL of 0.05 M HCl in evaporation flasks and transferred to a CE resin column. The flasks were rewashed with 4 mL of 0.01 M HCl, and the supernatant solution was transferred into the same resin column. This column was washed with 25 mL of 0.1 M oxalic acid to remove metals. Finally, the column was washed with 5 mL of 0.01 M HCl and 5 mL of DI water and then treated with 25 mL of 2.5 M NH₄OH. The resulting solution was collected in rotary evaporation flasks and dried at 45°C. The samples were then solubilized with 4 mL of 0.1 M HCl, transferred into centrifuge tubes, centrifuged for 15 min at 4200 × *g*, and poured into 5 mL vials. After centrifugation, the sample solutions were completely frozen (–18°C, >3 h) along

with 175 µL of the second internal standard (containing ca. 70 µg of d-methionine) and then dried. Then, the freeze-dried samples were placed in a methanol bath at 0°C and mixed with freshly prepared isopropanol (1:2.5, v/v) and 400 µL of 4 M HCl. Each vial was firmly stoppered with a Teflon-laminated septum, heated for 30 min at 110°C, and cooled to ambient temperature. The supernatant solution was transferred to smaller vials (2 mL). Then, 130 µL each of dichloromethane and pentafluoropropionic anhydride were mixed into the processed samples, and the vials were heated again for 10 min at 110°C. After cooling to ambient temperature, the solution was dried under an N₂ stream. The dried amino acids were dissolved in 150 µL of dichloromethane and shaken for 30 min. The supernatant solutions were then transferred into GC autosampler vials.

Amino acids were analyzed on a GC–MS instrument (QP 2010 PLUS; Shimadzu, Japan) coupled with a flame ionization detector. A 30 m capillary column (25 m × 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 the carrier gas, and the system was driven under the constant column and make-up-flow conditions (25 mL min^{–1}). The temperature was programmed as described by Zhang & Amelung (1996).

Statistical analysis

Before statistical analysis, the normality of the data frequency distribution for all the variables was tested by using the Kolmogorov–Smirnov test (Massey, 1951). Natural logarithmic transformation was used for non-normally distributed data. Statistically significant differences among treatments were tested by using a one-way analysis of variance followed by the least significant difference test (Sokal & Rohlf, 1995). All statistical analyses were performed with SPSS (SPSS Inc., Chicago, USA), and the figures were generated by using Origin Pro 8.5 software (Origin Lab Corp., Northampton, USA).

Results

Soil organic carbon (SOC) and its effect on total nitrogen (TN) and pH: After 23 years of long-term organic and inorganic fertilization, the OC contents in CM (12.20 C kg^{–1}) plots were significantly higher than those in HCM (9.28 g C kg^{–1}) and NPK (7.00 g C kg^{–1}) plots (Table 1). The OC contents (4.42 C kg^{–1}) in the control plot were the same before and after long-term fertilization. The OC content under different fertilization treatments followed the rank of CM > HCM > NPK > control. The TN content in the control soil plot was 0.47 g N kg^{–1} and was significantly lower than that in plots treated with other soil amendments, i.e., CM (1.56 g N kg^{–1}), HCM (1.10 g N kg^{–1}), and NPK (0.80 g N kg^{–1}). The pH values of the plots treated with organic and inorganic fertilizer amendments were slightly lower (8.01–8.58) than those of the control treatment, which had the highest pH value of 8.61.

Table 1. Soil properties after the use of compost and inorganic fertilizers for 23 years.

Treatments	SOC (g C kg ⁻¹)	Total nitrogen (g N kg ⁻¹)	pH	Mass proportion of aggregates (%)			Organic C in aggregates (g C kg ⁻¹)		
				M	m	SC	M	m	SC
CM	12.20±0.08a	1.56±0.06a	8.01b	30.8±3.2a	55.1±5.1c	14.1±2.0c	12.17±0.13a	8.80±0.25a	10.51±0.22a
HCM	9.28±0.08b	1.10±0.05b	8.19b	17.7±0.2b	62.9±6.1b	19.4±6.3b	11.80±0.04b	7.59±1.60a	7.32±0.11b
NPK	7.00±0.04c	0.80±0.04cd	8.17b	8.8±0.4d	70.1±2.8a	21.1±2.4b	11.68±0.27b	5.04±0.12b	5.77±0.07c
NP	6.64±0.05c	0.76±0.03cd	8.01b	10.1±0.4d	68.5±4.5a	21.4±4.1b	10.97±0.07c	4.81±0.06b	4.02±0.02e
PK	5.80±0.10d	0.62±0.04de	8.35a	14.8±0.7c	63.1±6.9b	22.1±7.6b	8.92±0.11d	4.82±0.02b	5.06±0.02d
NK	5.22±0.06e	0.88±0.05c	8.58a	9.9±0.1d	61.9±2.7b	28.2±2.7a	8.66±0.32d	3.66±0.05b	3.43±0.03f
Control	4.42±0.03e	0.47±0.04e	8.61a	8.8±1.2d	71.5±1.5a	19.7±0.2b	6.63±0.06e	4.22±0.06b	3.15±0.03g

CM: Compost, HCM: Combination of half compost plus half inorganic fertilizer N, NPK: Inorganic nitrogen-phosphorus-potash, NP: Inorganic fertilizer, PK: Inorganic fertilizer, NK: Inorganic fertilizer, Control: No fertilizer, SOC: Soil organic carbon, m: macro-aggregates, m: micro-aggregates, SC: Silt plus clay fraction Mean ± standard error (n = 4), Values within the same column followed by different letters indicate significant differences at $p < 0.05$

Mass proportion and OC content of soil aggregates:

The mass ratios of the micro aggregates in the CM, HCM, and NPK plots were 55.1%, 62.9%, and 70.1%, respectively, and were significantly ($p \leq 0.05$) lower than those in the control plot (71.5%) (Table 1). In contrast to the control plot, the CM and HCM plots were dominated by macro-aggregates, and the mass ratio of aggregates and small aggregates in these plots had increased by 30.8% and 17.7%, respectively. However, the micro aggregates and the powder-sand components in the CM and HCM plots were significantly ($p \leq 0.05$) reduced.

The OC contents of the micro aggregates under the control, CM, and NPK treatments were 4.22, 8.80, and 7.59 g C kg⁻¹, respectively. The OC contents of the SC fraction under the CM, HCM, and NPK treatments were 10.51, 7.32, and 5.77 g C kg⁻¹, respectively, which were significantly higher than those under other soil amendments (Table 1). The OC content in the aggregates under CM treatment (12.17 g C kg⁻¹) had increased relative to that in the aggregates under the control treatment (6.63 g C kg⁻¹). The said increase was mainly shown by macro- and micro aggregates. For macro-aggregates, micro-aggregates, and SC particles, the least values of soil organic content were obtained in NK treated soils while the highest ratios attained in CM treatments ranged from 8.66 to 12.17, 3.66 to 8.80, and 3.43 to 10.51 g C kg⁻¹, respectively.

L-Amino acids: Fourteen out of 22 L-amino acids were detected under all treatments (Table 2). Under the CM treatment, the most abundant L-amino acids were L-aspartic acid, L-alanine, and L-lysine with the corresponding contents of 21.83, 18.85, and 16.06 µg g⁻¹, respectively. However, under the HCM treatment, L-leucine (10.61 µg g⁻¹) had the highest content, followed by L-phenylalanine (10.03 µg g⁻¹) and L-valine (9.92 µg g⁻¹). Under all organic fertilization treatments, L-aspartic acid accounted for the highest L-amino acid content. Under NPK treatment, L-serine accounted for the highest L-amino acid content (13.92 µg g⁻¹), followed by L-proline (12.98 µg g⁻¹), L-isoleucine (12.61 µg g⁻¹), and L-lysine (11.12 µg g⁻¹). Under NP treatment, L-alanine

showed the highest value of 4.32 µg g⁻¹. L-glutamine was present at the concentration of 4.39 µg g⁻¹ in the soil under PK treatment. The concentration of L-glutamic acid was higher under organic treatment (10.12 µg g⁻¹) than under inorganic treatment (6.87 µg g⁻¹) and was higher than that of other amino acids under NK treatment. Compared with the soils under treatment with other amendments and the control, the soil amended with CM exhibited a significant ($p \leq 0.05$) increase in L-aspartic acid content, followed by L-alanine and L-lysine contents. Therefore, the CM was found as the best treatment compared to all other soil amendments.

D-amino acids: The D-amino acid contents varied with the different soil amendments (Table 3). Overall, 14 out of 22 D-amino acids were detected. The increase in D-aspartic acid content under other soil amendments followed the order of CM (11.95 µg g⁻¹) > HCM (7.31 µg g⁻¹) > NPK (5.51 µg g⁻¹). Under CM soil amendment, the most abundant D-amino acids were D-aspartic, D-alanine, D-proline, D-tryptophan, D-glutamic acid, D-phenylalanine, D-leucine, D-valine, and D-glutamine with the corresponding contents of 11.95, 11.21, 10.74, 10.28, 9.08, 8.86, 8.66, 8.49, and 8.37 µg g⁻¹, respectively. Under HCM treatment, the content of D-aspartic acid (7.31 µg g⁻¹) was the highest, followed by the contents of D-lysine (7.20 µg g⁻¹) and D-serine (7.16 µg g⁻¹). The D-aspartic acid content was the highest under CM treatment. Compared with the control treatment, soil fertilization significantly increased the contents of other amino acids, including D-glutamic, D-alanine, D-phenyl-alanine, D-glutamine, D-serine, D-valine, D-proline, D-leucine, D-isoleucine, D-tyrosine, D-tryptophan, D-asparagine, and D-lysine. The D-valine (9.85 µg g⁻¹), and D-tyrosine (8.30 µg g⁻¹) contents were significantly increased under NPK soil treatment. Likewise, D-serine (10.94 µg g⁻¹), and D-asparagine (8.15 µg g⁻¹) contents had also increased under NP application relative to those under the control treatment and all other amendments. Among all treatments, CM followed by HCM was the most effective for enhancing the D-amino acids.

Table 2. Influence of compost and synthetic fertilizers on L-amino acids.

Treatments	L-Aspartic acid	L-Glutamic acid	L-Alanine	L-Phenyl alanine	L-Glutamine	L-Serine	L-Valine
CM	21.83 ± 0.968a	10.12 ± 0.65a	18.85 ± 0.38a	6.78 ± 0.02b	6.68 ± 0.001	5.65 ± 0.006c	4.55 ± 0.009c
HCM	8.02 ± 0.02b	6.87 ± 0.01b	7.94 ± 0.004b	10.03 ± 0.25a	6.74 ± 0.01b	7.84 ± 0.03b	9.92 ± 0.05a
NPK	4.98 ± 0.005c	5.14 ± 0.01c	5.74 ± 0.006c	5.12 ± 0.006c	5.07 ± 0.004c	13.92 ± 0.04a	5.02 ± 0.007c
NP	0.41 ± 0.006e	3.29 ± 0.01d	4.32 ± 0.005d	3.13 ± 0.01d	3.01 ± 0.01d	4.18 ± 0.01d	2.92 ± 0.01d
PK	1.51 ± 0.01e	0.45 ± 0.04e	1.27 ± 0.01e	0.43 ± 0.01e	4.39 ± 0.01d	1.08 ± 0.007e	0.37 ± 0.006e
NK	0.01 ± 0.003f	4.78E-04 ± 0.003f	0.01 ± 0.002f	4.56E-04 ± 0.001f	4.10E-04 ± 0.008f	0.008 ± 0.001f	3.47E-04 ± 0.0004f
Control	0.001 ± 0.000009f	0.014 ± 0.007f	0.001 ± 0.003f	5.72E-06 ± 0.00004f	5.37E-06 ± 0.002f	0.001 ± 0.00004f	4.78E-06 ± 0.00006f
Treatments	L-Proline	L-Leucine	L-Isoleucine	L-Tyrosine	L-Tryptophan	L-Asparagine	L-Lysine
CM	5.60 ± 0.007c	9.67 ± 0.02a	5.58 ± 0.006c	9.48 ± 0.01a	12.30 ± 0.05a	9.28 ± 0.03a	16.06 ± 0.05a
HCM	7.67 ± 0.01b	10.61 ± 0.01a	7.56 ± 0.009b	6.54 ± 0.01b	7.41 ± 0.008b	6.46 ± 0.01b	5.68 ± 0.004c
NPK	12.98 ± 0.02a	5.81 ± 0.03c	12.61 ± 0.05a	4.93 ± 0.02c	5.55 ± 0.009c	4.84 ± 0.009c	11.12 ± 0.04a
NP	4.12 ± 0.003d	2.88 ± 0.009d	4.02 ± 0.01d	2.78 ± 0.03d	3.97 ± 0.005d	2.73 ± 0.02d	4.26 ± 0.007d
PK	0.98 ± 0.01e	0.33 ± 0.01e	0.89 ± 0.009e	0.31 ± 0.005e	0.84 ± 0.007e	0.28 ± 0.004e	1.18 ± 0.02e
NK	0.008 ± 0.0002f	2.79E-04 ± 0.0004f	0.007 ± 0.0002f	1.92E-04 ± 0.00004f	4.38E-03 ± 0.00006f	1.44E-04 ± 0.00008f	0.01 ± 0.001f
Control	0.001 ± 0.00003f	4.04E-06 ± 0.00004f	0.001 ± 0.00001f	3.36E-06 ± 0.0002f	4.81E-04 ± 0.001f	2.86E-06 ± 0.00007f	0.001 ± 0.0003f

CM: Compost, HCM: Combination of half compost plus half inorganic fertilizer N, NPK: Inorganic nitrogen -phosphorus-potash, NP: Inorganic fertilizer, PK: Inorganic fertilizer, NK: Inorganic fertilizer, Control: No fertilizer, Mean ± standard error (n = 4), Values within the same column followed by different letters indicate significant differences at p<0.05

Table 3. Influence of compost and synthetic fertilizers on D-amino acids.

Treatments	D-Aspartic acid	D-Glutamic acid	D-Alanine	D-Phenyl alanine	D-Glutamine	D-Serine	D-Valine
CM	11.958 ± 0.03a	9.0877 ± 0.01a	11.213 ± 0.017a	8.8626 ± 0.01a	8.4930 ± 0.032a	3.7324 ± 0.005d	8.3753 ± 0.05a
HCM	7.3126 ± 0.006b	6.3778 ± 0.011b	4.7317 ± 0.0068c	0.0165 ± 0.003e	2.3056 ± 0.022d	7.1620 ± 0.011b	6.0299 ± 0.007b
NPK	5.5183 ± 0.003c	4.7845 ± 0.0052c	5.4731 ± 0.012c	7.2565 ± 0.005b	4.5973 ± 0.006c	5.3782 ± 0.02c	9.8576 ± 0.17a
NP	3.9055 ± 0.04d	2.6272 ± 0.021d	3.19E-03 ± 0.00003f	2.5480 ± 0.007d	6.1177 ± 0.02b	10.941 ± 0.024a	1.9743 ± 0.03d
PK	0.7974 ± 0.005e	0.2380 ± 0.0025e	0.7319 ± 0.0046e	0.1726 ± 0.009e	6.2853 ± 0.013b	0.6048 ± 0.0085e	2.47E-03 ± 0.009e
NK	3.47E-03 ± 0.00006f	1.22E-04 ± 0.00068f	3.8729 ± 0.017d	1.07E-04 ± 0.00003f	2.20E-07 ± 0.000006e	9.81E-04 ± 0.00001f	9.08E-06 ± 0.00001e
Control	4.27E-04 ± 0.0003f	2.32E-06 ± 0.0002f	2.36E-04 ± 0.0000002f	2.11E-06 ± 0.0003f	1.34E-07 ± 0.000001e	1.46E-04 ± 0.000001f	1.34E-07 ± 0.000001e
Treatments	D-Proline	D-Leucine	D-Isoleucine	D-Tyrosine	D-Tryptophan	D-Asparagine	D-Lysine
CM	10.748 ± 0.045a	8.6605 ± 0.018a	6.6317 ± 0.007b	5.9760 ± 0.018b	10.284 ± 0.01a	3.4049 ± 0.035cd	7.904 ± 0.03b
HCM	7.091 ± 0.012b	6.2133 ± 0.01b	6.992 ± 0.014b	4.5026 ± 0.011c	6.926 ± 0.008b	5.9317 ± 0.008b	7.2055 ± 0.004b
NPK	5.300 ± 0.01c	4.6807 ± 0.0024c	5.224 ± 0.009c	8.3038 ± 0.036a	5.173 ± 0.007c	4.4379b ± 0.0012bc	5.4177 ± 0.008c
NP	3.636 ± 0.009d	2.4337 ± 0.018d	3.517 ± 0.041d	1.7986 ± 0.024d	3.460 ± 0.008d	8.1590 ± 0.02a	3.8333 ± 0.014d
PK	0.543 ± 0.019e	0.0534 ± 0.001e	0.504 ± 0.0006e	2.04E-03 ± 0.01e	0.477 ± 0.0002e	1.6612d ± 0.0002de	0.6751 ± 0.005e
NK	0.004 ± 0.000012f	5.44E-05 ± 0.0000009f	0.001 ± 0.0000007f	5.82E-06 ± 0.000008e	0.023 ± 0.0000002f	2.09E-03 ± 0.00014e	1.51E-03 ± 0.00002f
Control	0.001 ± 0.0000001f	2.94E-07 ± 0.000007f	5.44E-05 ± 0.000000f	1.27E-07 ± 0.000001e	0.001 ± 0.0000001f	1.19E-07 ± 0.0005e	2.36E-04 ± 0.000001f

CM: Compost, HCM: Combination of half compost plus half inorganic fertilizer N, NPK: Inorganic nitrogen -phosphorus-potash, NP: Inorganic fertilizer, PK: Inorganic fertilizer, NK: Inorganic fertilizer, Control: No fertilizer, Mean ± standard error (n = 4), Values within the same column followed by different letters indicate significant differences at p<0.05

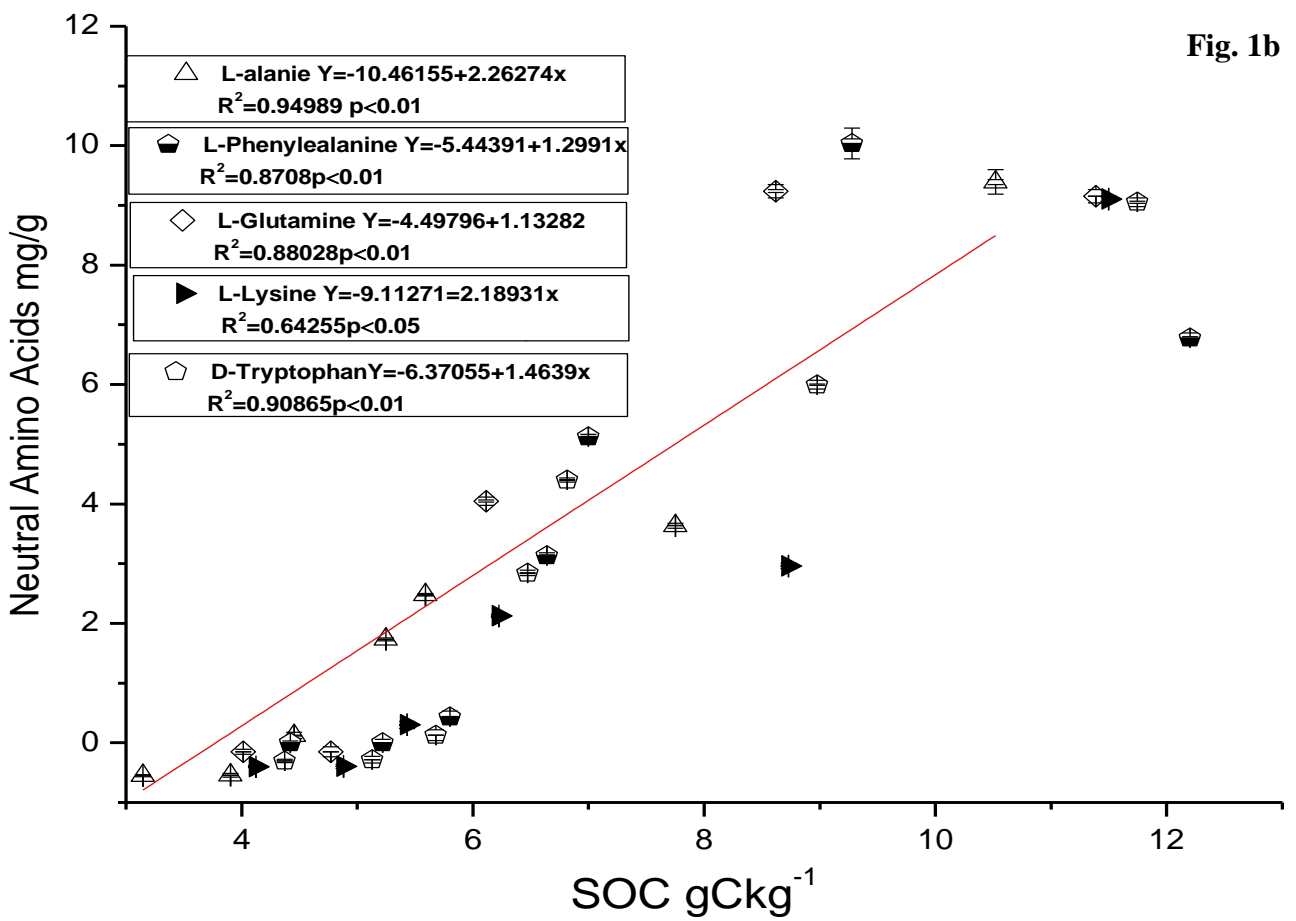
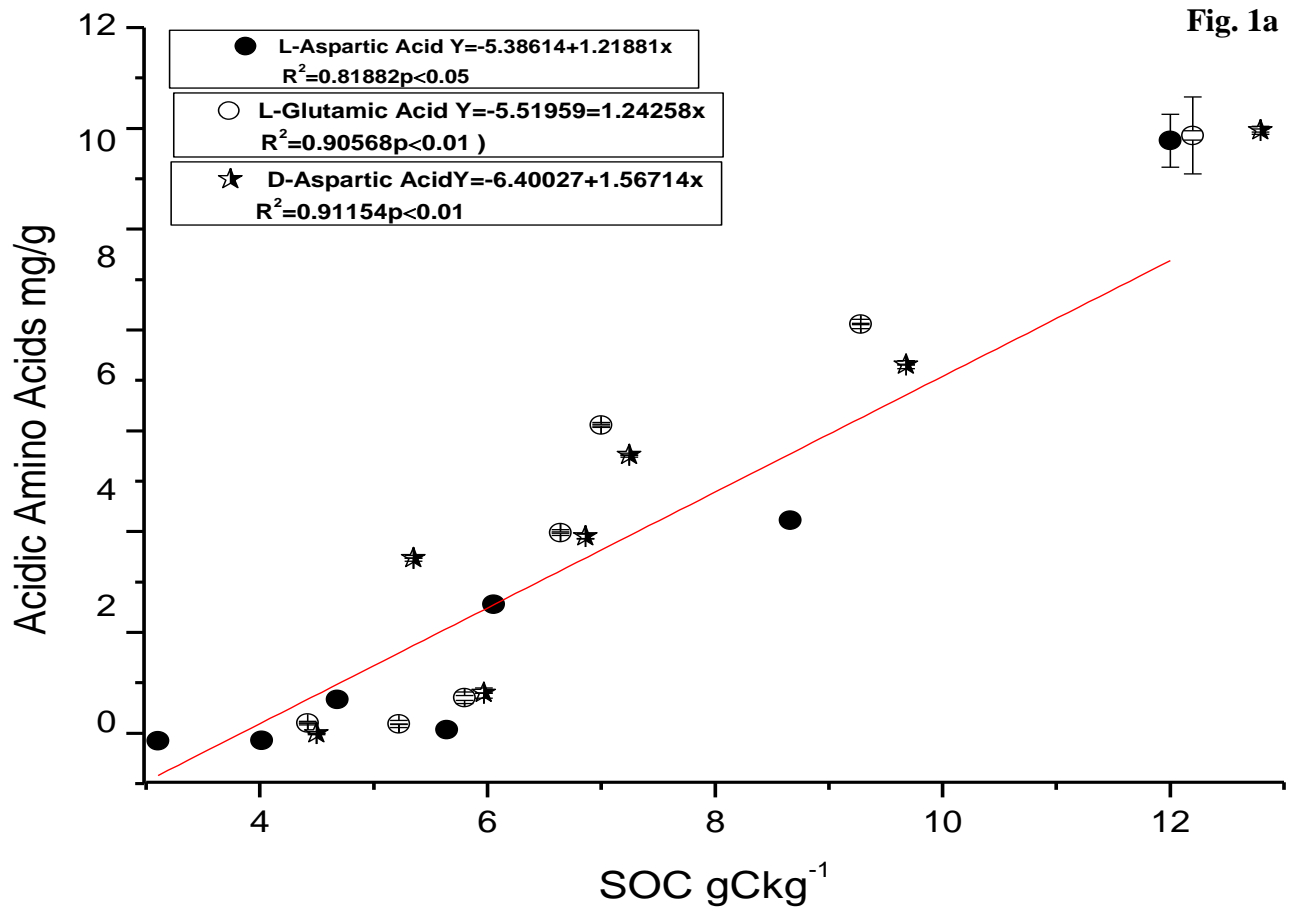


Fig. 1a, b. Correlation of SOC with acidic and neutral amino acids.

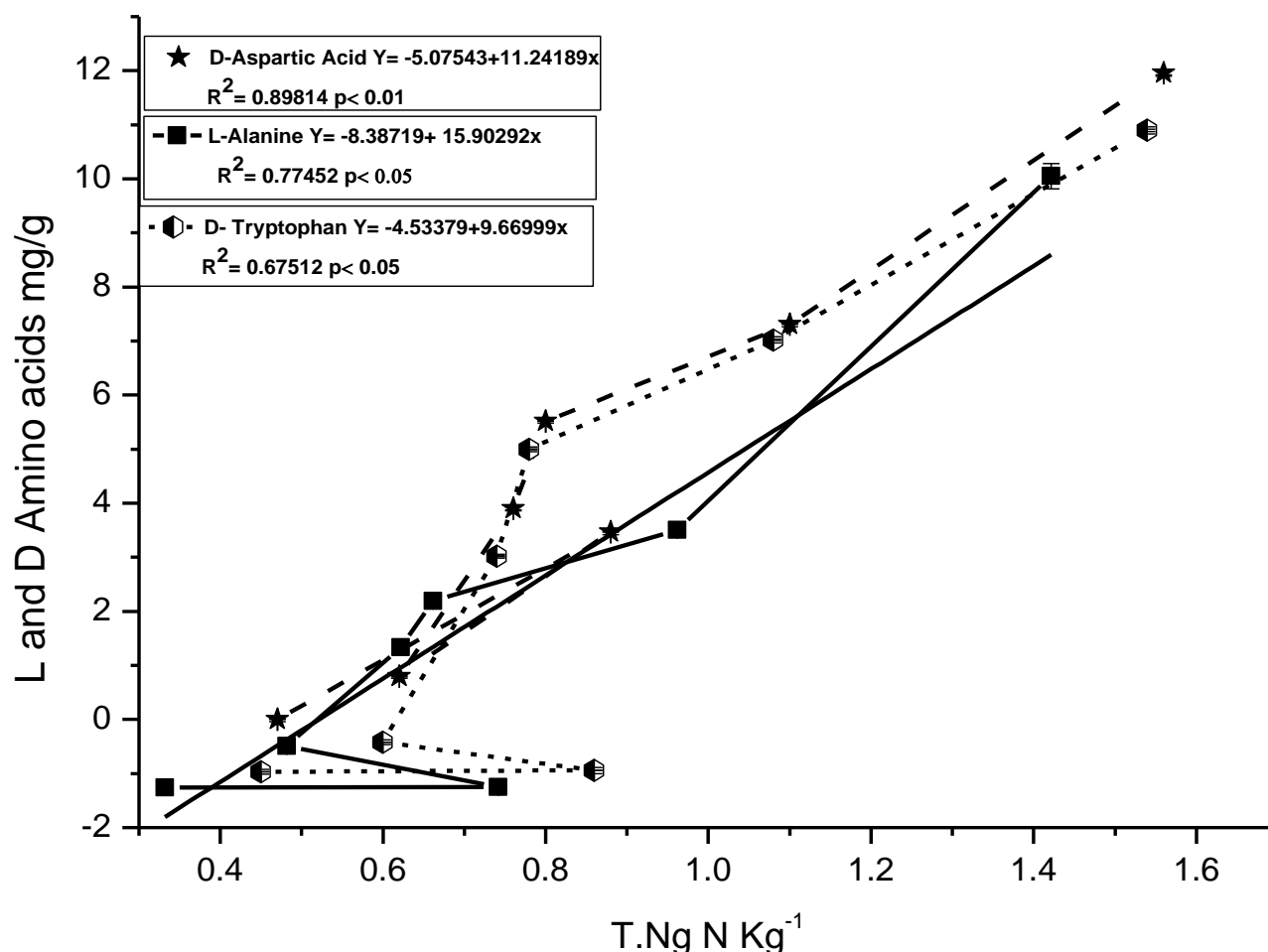


Fig. 2. Relationship of total nitrogen with L- and D-amino acids.

Correlation of L- and D-amino acids with soil properties: Correlation analysis revealed that L-aspartic, L-glutamic, and D-aspartic acids were significantly ($p \leq 0.01$) and positively associated with SOC with R^2 values ranging from 0.81 to 0.91 (Fig. 1a). Similarly, L-alanine, L-lysine, L-phenyl-alanine, L-glutamine, and D-tryptophan were significantly ($p \leq 0.01$, 0.05) and positively correlated with SOC content ($R^2 = 0.64$ – 0.94) (Fig. 1b). Moreover, L-alanine showed a significant ($p \leq 0.05$) positive association ($R^2 = 0.77452$) with TN (Fig. 2). The mass proportion of macroaggregates demonstrated a significant ($p \leq 0.05$) positive association with L-aspartic acid, L-glutamic acid, L-alanine, D-aspartic acid, and D-tryptophan (Fig. 3a). The mass proportion of micro aggregates showed a highly significant positive correlation with L-glutamine and a significant correlation with L-serine (Fig. 3b).

Correlation analysis further revealed that D-tryptophan, D-aspartic acid, and L-alanine had a significant positive correlation with TN ($p \leq 0.05$, $R^2 = 0.77$; $p \leq 0.05$, $R^2 = 0.67$ – 0.89) (Fig. 2). The mass proportion of micro aggregates showed a significant ($p \leq 0.01$) positive correlation ($R^2 = 0.99$) with D-tryptophan, D-aspartic acid, and D-asparagine (Fig. 3b). The D-amino acids and SC fractions also showed a significant ($p \leq 0.01$) correlation with D-aspartic acid, D-tryptophan, and D-asparagine (Fig. 3c).

Discussion

The long-term use of organic and synthetic fertilizers has an important role in the accumulation of SOC, soil aggregates, and L- and D-amino acids. Amino acids can be divided into three groups, i.e., acidic, basic, and natural. L- and D-amino acids are present in soil organic matter (SOM), and the mineralization of D-amino acids is slower than that of L-enantiomers. Plants can directly take up D-amino acids through their roots but utilize a very small quantity of D-amino acids. The main aim of the present research was to determine the route of L- and D-amino acids in the soil and the consumption of these amino acids by soil microorganisms to provide suggestions for future research. Therefore, determining the importance of L- and D-amino acids for crop growth and production in different managed and unmanaged ecosystems is essential (Bibi & Ding, 2018).

The results of this work revealed the abundance of several acidic, basic, and neutral amino acids. L- and D-aspartic acid contents were higher under CM treatment than under NPK treatment and other soil amendments. Past findings have also revealed that the acidic amino acids were decreased under non-organic nitrogen application, proving that synthetic fertilizers reduced the accumulation of L- and D-amino acids (Malhi *et al.*, 2003a; Bibi *et al.*, 2018). However, the use of N fertilizers significantly affected the amino acid concentrations (Reis *et al.*, 2020). Basic amino acids, i.e., L-lysine, tended to accumulate more under NPK

treatment than under HCM treatment at amounts less than those under CM treatment. The pattern of L-lysine accumulation contradicted that of D-lysine, which tended to accumulate less under NPK than under HCM and CM soil amendments. These findings were also inconsistent with past findings showing that the amounts of basic amino acids (lysine) remain constant under various soil amendments (Malhi *et al.*, 2003b; Creamer and Filley, 2013). The present findings confirmed that D- and L-alanine were higher under CM treatment than under other treatments. Previous findings have demonstrated that alanine is more important for soil fertility than other amino acids (Andersson & Berggren, 2005). Jones *et al.*, (2005) reported that amino acids dominate the organic nitrogen pool that is readily utilized by crop plants. In contrast to that under long-term fertilization, the number of basic amino acids tends to decrease under short-term fertilization; this result indicated that organic nitrogen is more stable than inorganic nitrogen (Nordin *et al.*, 2001; Brodowski & Amelung, 2005). The use of organic manure, such as compost, strengthens the mutual synergistic effect between the enzymes involved in the carbon cycle and macroaggregates that is highly beneficial for SOC sequestration (Zhanhui *et al.*, 2018; Zhao *et al.*, 2018; Bibi *et al.*, 2019).

Under CM soil amendment, the amounts of natural amino acids increased significantly in the following order: L-alanine > HCM, L-phenyl alanine, L-valine, L-leucine > NPK L-serine, L-proline, L-isoleucine. These results suggested that amino acids were the basic source of soil organic nitrogen with the maximum amount of organic matter. L- and D-glutamic acids also showed the highest values under CM and HCM treatment. Glutamic acid is the only amino acid that increases significantly after the addition of straw (Perez & Zhang, 2015). In addition, a high concentration of glutamic acid is correlated with organic matter. Similarly, the ratios of the L- and D-amino acids glutamine and glutamic acid can be an indicator of acid stress at the community levels (O'Dowd *et al.*, 1997; Zhao *et al.*, 2020). L-proline tended to accumulate under NPK fertilization, whereas D-proline showed the highest values under CM amendment. Proline is a root-loving amino acid, and it can be easily utilized by soil microorganisms and green plants due to its slow rate of metabolism in the soil (Lipson *et al.*, 2001).

Amino acids are one of the largest identifiable groups of organic N compounds in soils (Hopkins *et al.*, 1997). D-amino acids are far less abundant in nature than L-amino acids. L- and D-amino acids enter the soil from different sources, including plant, animal, and microbial biomass and feces (Andersson *et al.*, 2001; Andersson & Berggren, 2005). Moreover, D-amino acids occur in the soil due to the abiotic or biotic racemization of L-amino acids, and the degradation of D-amino acids is slower than that of L-amino acids (O'Dowd *et al.*, 1997). L- and D-amino acids are bound to SOM or soil colloids and also occur as free amino acids that are dissolved in the soil solution (Andersson & Berggren, 2005). Plants can directly take up D-amino acids by their roots but can only utilize a small quantity of D-amino acids; this limitation inhibits plant growth (Hopkins *et al.*, 1997). Microbial death also provides free amino acids that plants are capable of taking up (O'Dowd *et al.*, 1997).

This study showed that L- and D-tryptophan accumulation was highest in CM-treated soil but was lower than L- and D-alanine accumulation. High amounts

of L- and D-serine were found in NP-treated soil. Perez & Zhang (2015) identified serine as one of the most abundant amino acids in forest and agricultural soils. Physical processes, such as the sorption of amino acids into the soil matrix, play an important role in free amino acid content and strongly affect the structure of sandy and acidic forest soils. They also reported that organic soils contain high concentrations of free amino acids, i.e., histidine and lysine. The addition of urea (inorganic fertilization) altered the composition of several free amino acids (D-amino acids) in soil. Tryptophan is a neutral amino acid and is thus not preferentially absorbed despite its abundance in the soil (Perez & Zhang, 2015). Most of the accumulated proline in plants is transferred without metabolic conversion. The higher conversion of precursors into D-glutamine and D-alanine than into L-amino acids may be due to the accumulation of organic nitrogen in bacterial cell walls (Creamer & Filley, 2013). Roots successfully compete with microorganisms for free amino acids in the soil, and woody cluster soils have higher amounts of valine, proline, and serine.

In the present research work, the residual effects of various combinations of compost and inorganic fertilizer inputs on the biochemical, biological, and physicochemical attributes of arable fields were assessed. SOC contents under CM amendment were the highest, followed by those under HCM treatment. The TN contents in soils under CM and HCM amendments were higher than those under NPK, NP, NK, PK, and control treatments. These results were in line with past findings showing that biomass carbon and other nutrients (D-amino acids) under amendment by farmyard manure are the highest, followed by those under NPK treatment, and those under the control treatment are the lowest (Hopkins *et al.*, 1997; Malhi *et al.*, 2005). Moreover, the C and N contents in soil are attributed to the presence of crop residues, which in turn is associated with crop yield (Malhi *et al.*, 2003c). The pH values under all treatments were slightly lower than those under control, followed by those under NK and PK soil amendments. The lowest pH value of 8.01 was observed under CM and NP treatments. Low soil pH may be considered favorable for the accumulation of acidic and neutral amino acids.

SOM is firmer than clay and is more likely than macro aggregates to promote microbial degeneration (Andersson & Berggren, 2005; Zhao *et al.*, 2018). The results of the present study showed that macroaggregates were significantly increased with the application of HCM and were lowest under CM and NP treatments. Organic manure amendment significantly enhanced OC concentrations in aggregates and aggregate sub-fractions (Bibi *et al.*, 2018, 2019; Zhao *et al.*, 2020). The micro aggregate proportions under CM- and NP-treated soils were higher than those under other treatments and the control. Consequently, the mass proportion of micro aggregates was highest under CM and inorganic fertilizer NP treatments. The free SC proportion significantly decreased under HCM and NPK treatment and showed a significantly negative correlation with L-aspartic acid and L-alanine contents. However, D-aspartic, D-tryptophan, and D-asparagine showed a significant positive correlation with the SC fraction. Therefore, as previously confirmed that the relationship of L- and D-amino acid contents with the aggregate fraction is not important in long-term agricultural practices (Brodowski & Amelung, 2005).

Fig. 3(a)

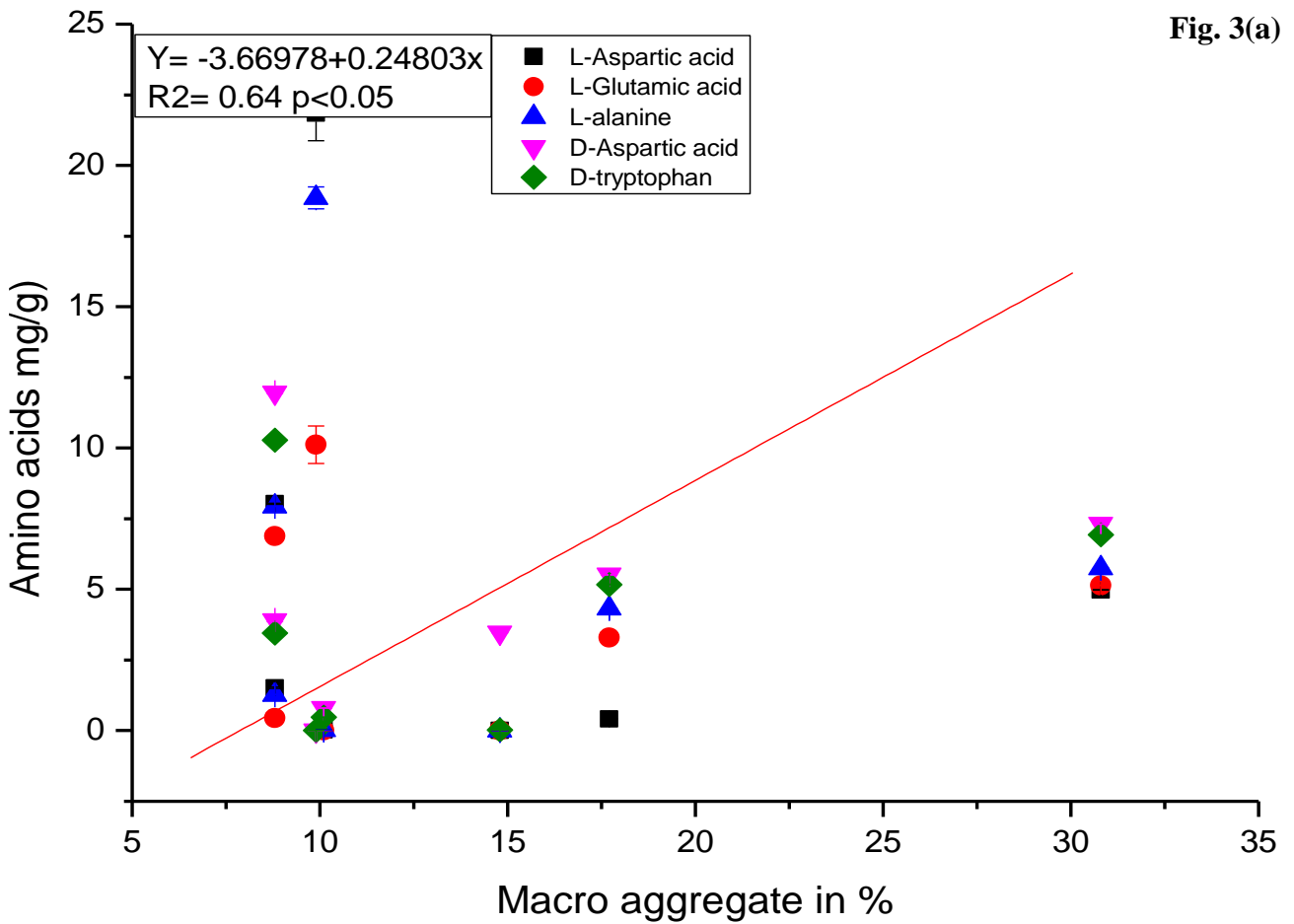
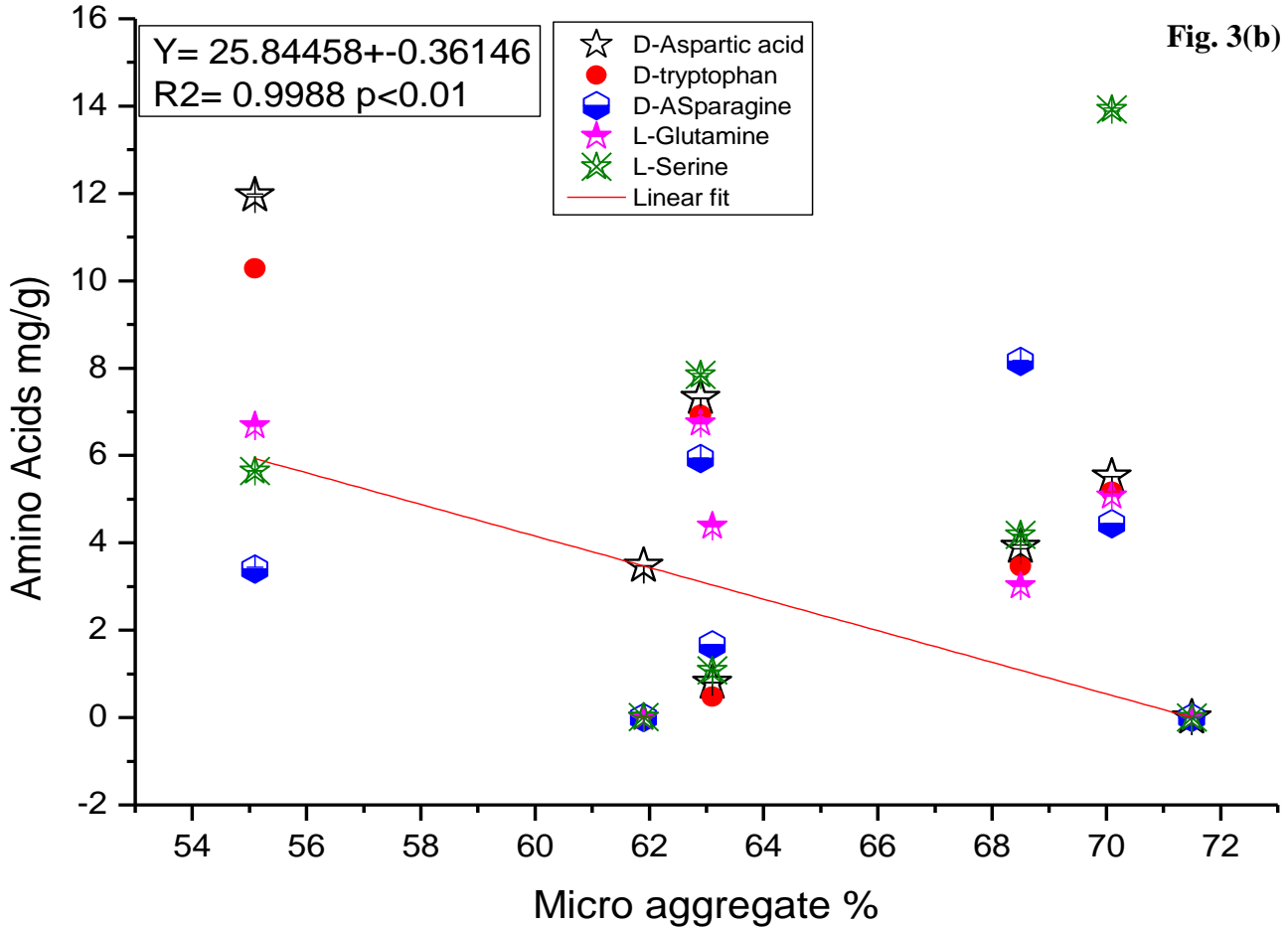


Fig. 3(b)



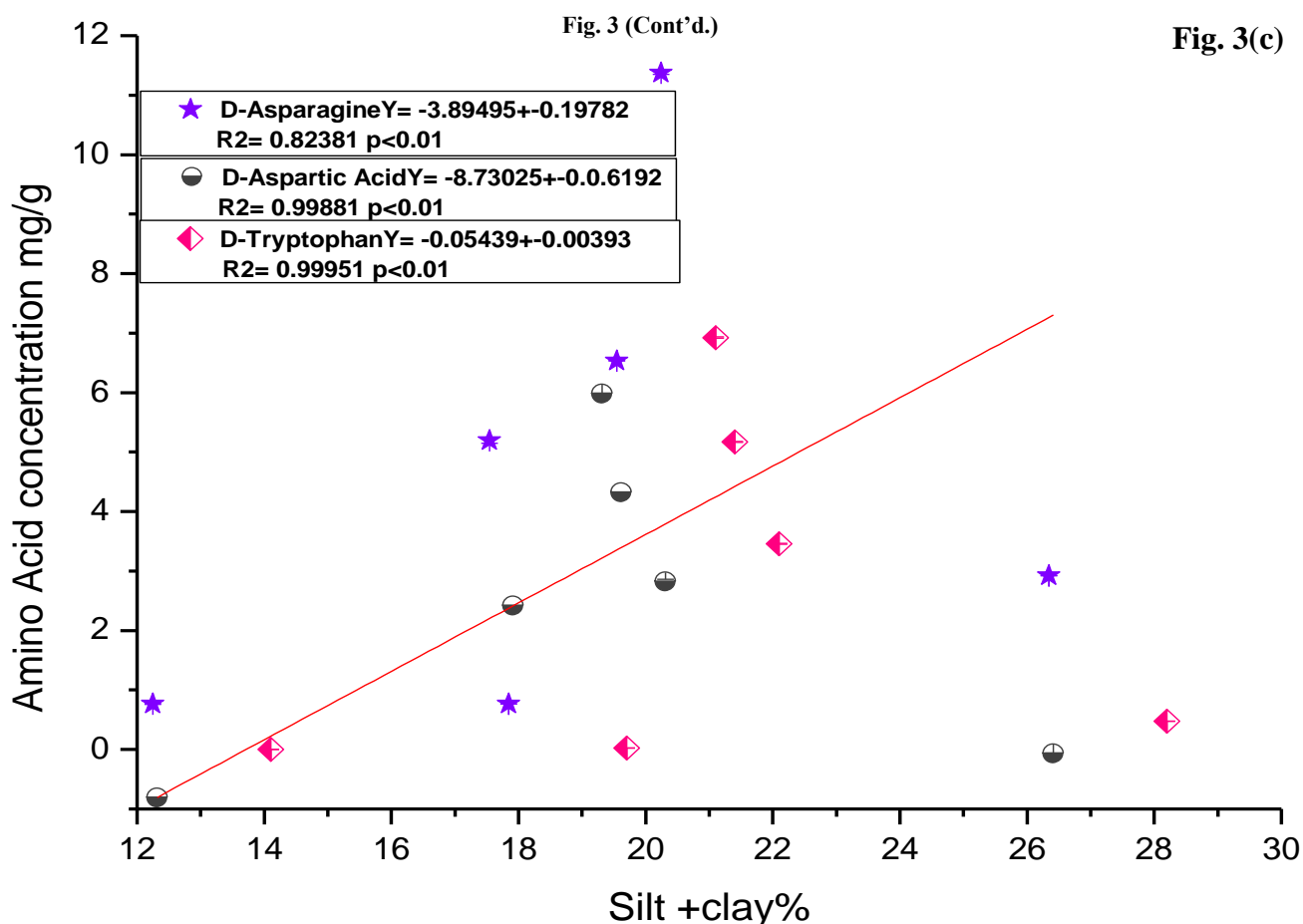


Fig. 3a, b, c. Correlation between mass proportion of aggregates with L- and D-amino acids.

In contrast to the control treatment, the prolonged application of organic and inorganic fertilizers had a positive effect on TOC and significantly increased SOC while decreasing soil bulk density (Chaudhary *et al.*, 2017; Zhao *et al.*, 2019). Organic manure significantly decreased soil bulk density and increased OC and N stocks in the soils of both systems. N and P fertilizers and manure have large effects on OC and N stocks in >2 mm aggregates in a continuous winter wheat system (Hao *et al.*, 2017). Fertilization significantly increased organic carbon contents in soil, macroaggregates, and the SC fraction, whereas compost application significantly reduced the specific activities per unit organic carbon in soil and aggregate sizes as compared with the control treatment (Yu *et al.*, 2012a, b; Bibi & Ding, 2018). Compared with other soil amendments and the control, CM amendment resulted in a significant increase in L-aspartic acid and then in L-alanine and L-lysine. Therefore, CM was recognized as the best amendment by comparing it with all other treatments. The amino acids glycine, glutamine/histidine, asparagine/serine, and aspartic acid have been previously identified as dominant amino acids (Andersson & Berggren, 2005). Malhi *et al.*, (2005) considered CM as an important factor for sustainability and improving soil quality and productivity. Similarly, the present results also indicated that among all other amendments, OM soil amendment followed by HCM treatment was the most effective for enhancing D-amino acid contents. However, Amelung *et al.*, (2006)

suggested that D-amino acids biexponentially decrease to approximately 30% under long-term cropping.

This study revealed that L-lysine had a significant positive correlation with SOC because the racemization of L-lysine is slower than that of other amino acids. D- and L-lysine are closely related to organic matter (Amelung *et al.*, 2001). Incubation studies on the pathway of organic nitrogen conversion showed that amino acids were increased with the enhancement in SOM. Therefore, further studies are needed to authenticate the role of organic nitrogen in various soil processes and quantify the utilization of organic nitrogen by different crop plants and soil microorganisms.

Conclusion

Under maize and wheat rotational cropping, long-term organic and inorganic fertilization through CM and HCM treatments provided a sizable pool of amino acids in the form of organic nitrogen that is necessary for increasing soil fertility. Soils under CM and HCM treatments contained significantly more SOC and organic nitrogen in the form of L- and D-amino acids than soils under inorganic fertilization amendments, i.e., NPK (alone or in combination). Given that amino acid concentrations under CM and HCM soil amendments were greater than those under synthetic fertilizers and control treatments, the application of organic fertilizer alone or in combination with NPK resulted in the significant accumulation of L- and D-amino acids.

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