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ORGANIC AND MINERAL FERTILIZERS SWAY THE NITROGEN AVAILABILITY TO PLANTS VIA MICROBIAL DIVERSITY – A LONG-TERM IMPACT

ZARINA BIBI[1,2,3*], WEIXIN DING[1*], GHULAM JILANI[4], NAQIB ULLAH KHAN[5*] AND HAFSA SALEEM[6]

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences (CAS), Nanjing - China
[2]University of Chinese Academy of Sciences (UCAS), Beijing - China
[3]Institute of Soil and Environmental Sciences, Facultuy of Agriculture, Gomal University, D.I. Khan, Pakistan
[4]Institute of Soil Science and Soil & Water Conservation, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[5]Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar, Pakistan
[6]Department of Agricultural Extension, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[*]Corresponding authors' email: wxding@issas.ac.cn; bibizarina100@yahoo.com; nukmarwat@yahoo.com

Abstract

Continued application of organic matter and inorganic synthetic fertilizers to soil influences the nutrient dynamics and diversity of microbial communities. A single site field study (plot size of 9.5 m × 5 m used in each treatment) encompassing 23 years compared the impact of organic and NPK fertilizers for exploring amino acids as indicators of nitrogen fate, and phospholipid fatty acids (PLFA) as biomarkers of microbial diversity in North China Plain field. Treatments in quadruplicated RCBD experiment were: whole N from organic manure (OM); half N from OM + half N from chemical fertilizer (HOM); chemical NPK fertilizers (NPK), NP, NK, PK, and no fertilizer control or check (CK) applied to raise wheat and maize crops continuously. To each crop, N was applied at the rate of 150 kg N ha^{-1} through organic manure and/or chemical fertilizer urea in two splits (90 + 60 kg N ha^{-1}). Calcium superphosphate (150 kg P_2O_5 ha^{-1}) and potassium sulfate (150 kg K_2O ha^{-1}) were applied basally in respective treatments. Soil samples were drawn from each treatment after 23 years of experimentation. These fertilization amendments exhibited that contents of total nitrogen and amino acids were significantly ($p \leq 0.05$) higher under OM and HOM compared to NPK and CK treatments. Total amino acids determination revealed that N and P included treatments among mineral fertilizers mainly contributed to amino acids residues in soil. The PLFA profiling exhibited that increased amino acids under OM and HOM correlated significantly ($p \leq 0.05$) and positively with an increase of Gram-positive (G+ve) bacteria and fungi, negatively with actinomycetes, and non-significantly with Gram-negative (G–ve) bacteria. These results suggest that accrual of organic and microbial residues (G+ve bacterial and fungal) enhance the N stabilization in surface soil, which ultimately could increase the plant growth and yield of crops.

Key words: Organic manure; Soil microbes; PLFA biomarkers; Plant nutrients; Amino acids.

Introduction

Incorporation of organic matter in to the croplands is imperative for increasing the productivity of soil, and to enhance its capacity for C and N storage (Wu *et al.*, 2018). Soil microorganisms influence the processes and mechanisms for the formation, stabilization, and transformation of soil organic carbon (SOC) owing to their role in organic matter (OM) turnover, nutrient cycling and microbial biomass production (Zhang *et al.*, 2015). Microbial residues play an enormous role in soil organic matter (SOM) accumulation and stabilization (Ding *et al.*, 2017). Use of organic manures and mineral / chemical fertilizers modifies the mode of SOC sequestration, that varies with the input source of organic carbon (Ding *et al.*, 2013; Ali *et al.*, 2018), and ultimately due to differences in the composition and performance of soil microbes (Stark *et al.*, 2007). Microbial residues (i.e., necromass) in soil exhibit longer storage time than their parent plant residues and other organic amendments (Throckmorton *et al.*, 2015). Contribution of microbial residues within SOM is now greatly acknowledged (Miltner *et al.*, 2012) since it is a principal source of stable C pool playing a significant role in C-sequestration endured in soils, than realized earlier (Liang *et al.*, 2011). Methodical options for tracking the fate / mechanisms of transforming the above-ground residues into SOM for increasing C and N stocks in cultivated soils need meticulous research based on mechanistic linkages between indicators and soil functions (Creamer *et al.*, 2016).

Amino acids are among the major sources for both C and N in soil; thus, signify a vital link between C and N cycles. The most abundant amino acids in soil are alanine and glutamic acid accounting for about 15 and 10%, respectively, of the amino acids recovered from dissolved organic carbon (DOC) (Fischer *et al.*, 2007). Amino acids as main compounds for soil organic nitrogen (SON), account for 20-50% of the soil N storage pool (Muruganandam *et al.*, 2009) for the immobilized N (Lü *et al.*, 2013), and are major source of available N for soil microorganisms and plants (Werdin-Pfisterer *et al.*, 2009). Inorganic N is quickly immobilized into microbial tissues, thus the newly synthesized amino acids are the principal compounds for C and N supply as well as storage in soil (Zhang *et al.*, 2015).

While microbial biomass ranges 2-3% of the total organic carbon (TOC) content in soil (Anderson & Domsch, 2010); nevertheless, its contribution can be >50% of total extractable SOM, about 45% of the humin fraction and >80% of soil N (Simpson *et al.*, 2007). Microbial SOM contains about 2% active microbes, while dormant and dead ones represent 42% and 56%, respectively (Blagodatskaya & Kuzyakov, 2013). For knowing about the modes of SOC and SON transformation for ultimate sequestration, therefore, requires modus operandi that generates knowledge and data on the microbial groups present, and their metabolic processes in the soil (Apostel *et al.*, 2013; Kanwal *et al.*, 2018).

Among the soil total nitrogen (TN) fractions, organic forms exceed 90% found as acid insoluble N and total hydrolysable N comprising mostly NH_4^+, amino acid and amino sugar N (Stevenson, 1994). The SON turnover is significantly influenced by elevated N pool, and its rate of enzymatic mineralization determines the N cycling and bioavailability; therefore, a decreased amino acid N content is obtained with N addition (Tian *et al.*, 2017). Microbial transformation of organic-N into microbial residues, and the effects of inorganic fertilizer inputs on this process have rarely been documented. Therefore, it was hypothesized that input of organic and/or mineral N fertilizers could have variable effect on nitrogen and amino acid residues contributing to N bioavailability to plants, and C and N stabilization in soil. Main objective of this long-term field study was to compare the impact of some organic and inorganic fertilizer treatments on soil microbial community structure, amino acid composition and the content of soil nitrogen available for plant growth.

Materials and Methods

Experimental site and design: This long-term field experiment was initiated in September 1989 at the Fengqiu State Key Agroecological Station, Fengqiu County, China (35°00′N, 114°40′E). Wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) have been grown there in winter and summer, respectively, as test crops. According to Zhang *et al.* (2015) the soil had sandy loam texture 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.

The experiment included seven treatments: whole amount of N (150 kg ha^{-1}) from organic manure (OM); half amount of N (75 kg ha^{-1}) from organic manure + half amount of N (75 kg ha^{-1}) from chemical fertilizer (HOM); chemical NPK (nitrogen-phosphorus-potash) fertilizer; chemical NP fertilizer; chemical NK fertilizer; chemical PK fertilizer; and no application of organic or chemical fertilizer (control or CK). These treatments were arranged in a randomized complete block design with four replicate plots (9.5 m × 5 m each). Nitrogen was applied in the form of urea at the rate of 150 kg N ha^{-1} to each crop in two splits: 60 kg N ha^{-1} as basal fertilizer, and 90 kg N ha^{-1} as supplemental fertilizer for maize; and 90 kg N ha^{-1} as basal fertilizer, and 60 kg N ha^{-1} as supplemental fertilizer for wheat in the NPK, NP and NK treatment plots. For HOM, 75 kg N ha^{-1} urea was used as a supplemental fertilizer for maize; while 15 and 60 kg N ha^{-1} as basal and supplemental fertilizer, respectively, for wheat. Calcium superphosphate (150 kg P_2O_5 ha^{-1} for NPK, NP and PK), potassium sulfate (150 kg K_2O ha^{-1} for NPK, NK and PK) and organic manure (1164 kg C ha^{-1} and 150 kg N ha^{-1} for OM treatment, and 582 kg C ha^{-1} and 75 kg N ha^{-1} for HOM treatment) were applied as the basal amendment.

Basal fertilizers and manure were applied on the soil surface, and the soil was plowed immediately before plantation of maize (early June) or wheat (early October). Supplemental fertilizers were also applied on the soil surface for wheat and maize crops in late February and late July, respectively, following rainfall or irrigation. Same field management practices were applied for all the treatment groups. Noticeable weeds were removed by hand. At harvest, the wheat and maize plants were removed completely from the field except for the stubbles and roots. After each crop, experimental field was tilled with normal cultivator for land preparation to grow the next crop.

Soil sampling and analysis: In September 2012, ten soil samples were collected from each treatment plot with 2.5 cm diameter auger up to the depth of 20 cm. Soil samples were thoroughly mixed to form one composite sample. Moist soil samples were gently broken apart along natural-break points and screened through an 8 mm sieve. Plant and organic debris in the sieved soil were carefully removed with forceps. After thorough mixing, soil moisture was measured by drying a sub-sample at 105°C. Another sub-sample was air-dried for the determination of soil properties. The remaining moist soil was used for wet-sieving. The wet oxidation method and Kjeldahl method were used to measure SOC and total nitrogen (TN), respectively (McGill & Figueiredo, 1993).

Microbial PLFA determination: Microbial PLFAs were extracted following a modified Bligh-Dyer technique (Brant *et al.*, 2006). For this, 3 g (on the oven-dried basis) of fresh soil samples were extracted with methanol-chloroform-phosphate buffer (2:1:0.8). Extracts were centrifuged at 1330 ×*g* for 10 min, and the chloroform phases were collected. Phospholipids were separated from glycolipids and neutral lipids using silicic acid-bonded solid-phase-extraction columns. Phospholipids were saponified, and the fatty acids were trans-methylated to the corresponding fatty acid methyl esters (FAMEs) under N_2 at 37°C, and then dissolved in hexane, which contained a methyl nonadecanoate (19:0) FAME standard. The resulting FAMEs were analyzed with GC-MS instrument (QP 2010 PLUS; Shimadzu, Japan).

Amino acids assay: Soil amino acids were measured by gas chromatograph (Zhang & Amelung, 1996). For this, 200 mg of the air-dried and sieved (≤0.25 mm) soil was hydrolyzed with 20 mL of 6 M HCl for 12 h (105°C). Then 200 µL internal standard solutions containing 80 µg L^{-1} norvaline was added to the hydrolysates (Amelung, 2001). Dissolved amino acids were added to the column. Evaporation flasks were washed again with 4 mL 0.01 M HCl, and the resulting solution was also added to the resin column. Amino acids were eluted with 25 mL of 2.5 M NH_4OH, collected in rotary evaporation flasks, and the supernatant was transferred to GC auto sampler vials. The GC analysis was carried out on a GC-MS instrument (QP 2010 PLUS; Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector.

Data processing and statistical analysis: Before statistical analysis, normality of data frequency distribution for all the variables was tested using Kolmogorov-Smirnov test. Natural logarithmic transformation was used for non-normally distributed data. Statistically significant differences among treatments were tested using one-way analysis of variance followed by least significant difference test (Sokal & Rohlf, 1997). All the statistical analyses were performed with SPSS (SPSS Inc., Chicago, USA), and figures were drawn by using Origin Pro 8.5 software (Origin Lab Corp., Northampton, USA).

Results

Soil nitrogen contents: Total N contents were statistically ($p \leq 0.05$) greater with OM and HOM if compared to NPK, NP and NK treatments that had non-significant difference mutually; however, all showed higher values than in control and PK treatments (Table 1). Nitrogen contents were higher in nitrate (NO_3^-) form as compared to ammonium (NH_4^+). Greater contents of NO_3^- –N were obtained from OM and HOM followed by NP and NPK fertilizer treatments, and the smallest value was in control soil. Data on NH_4^+ –N contents did not match with the nature of treatments, and it was not detected for HOM and fertilizer treatments of PK and NK. The NH_4^+ –N concentration was also greater for OM treatment followed by control, while NPK and NP treatments showed lower NH_4^+ –N content. These results did not reflect a clear impact of fertilizer treatments on NH_4^+ –N content in the soil.

Total amino acids: Among all the treatments soil samples, total amino acids were yielded differentially in significant amounts (Fig. 1). Soils amended with OM and HOM rendered a significant increase ($p \leq 0.05$) in the concentrations of total amino acids than yielded by the di- and tri-nutrient treatments of N, P and K fertilizers. Under the di-nutrient fertilizer treatments (NP, PK, NK), the amount of total amino acids was although very low but significantly greater than in control.

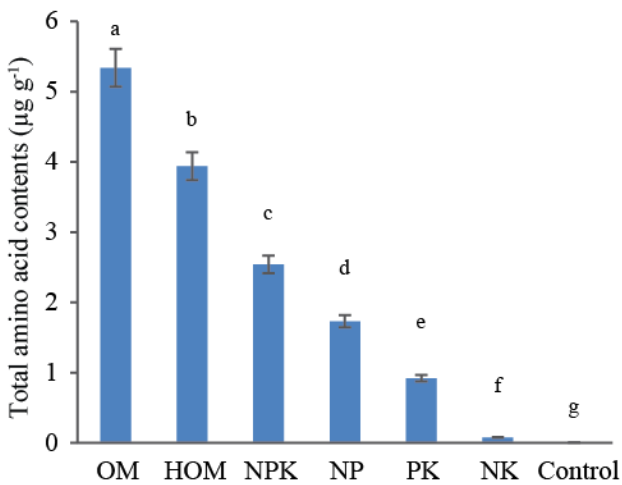

Fig. 1. Long-term influence of different organic and chemical fertilizer combinations on total amino acid contents in the soil. Treatments details: whole N from organic manure (OM); half N from organic manure + half N from inorganic fertilizer (HOM); mineral NPK fertilizers (NPK), NP, NK, PK, and no fertilizer (control).

PLFA profiles: Soils treated with different treatments revealed 42 PLFAs; however, 36 (each one accounting for at least 1% of the total PLFAs) were used for analysis (Tables 2 and 3). Concentrations of total PLFAs and bacterial PLFAs were significantly ($p \leq 0.05$) higher with HOM among all the treatments. It was followed by OM having corresponding PLFAs content, both treatments having significant difference for bacterial PLFAs. Both these amendments yielded PLFAs being statistically higher if compared to all other treatments. Non-significant differences were found between control and inorganic fertilizer treatments for most of the PLFA groups, except that for Gram-positive (G+ve) bacterial and actinomycetes PLFAs. Compared with control, manure application significantly ($p \leq 0.05$) increased the G+ve bacterial PLFAs, and reduced the actinomycetes PLFAs. However, manure application showed no effect on the Gram-negative (G−ve) bacterial PLFAs. Fungal PLFAs values across all the treatments were highest for HOM treatment, while the lowest was found in the NPK treatment. Among the mineral fertilizer treatments, NPK produced significantly higher content of G+ve bacterial PLFAs but lower content of fungal PLFAs if compared to di-nutrients NP, PK and NK fertilizer treatments.

Table 1. Long-term influence of different organic and chemical fertilizer combinations on various components of nitrogen in soil.

Treatments	Nitrate –N (mg kg^{-1})	Ammonium–N (mg kg^{-1})	Total–N (g N kg^{-1})
OM	18.26 a	0.93	1.56 ± 0.06 a
HOM	14.66 b	ND	1.10 ± 0.05 b
NPK	10.27 c	0.81	0.80 ± 0.04 cd
NP	12.41 c	0.69	0.76 ± 0.03 cd
PK	6.96 d	ND	0.62 ± 0.04 de
NK	6.78 d	ND	0.88 ± 0.05 c
Control	5.04 d	0.90	0.47 ± 0.04 e

Treatments comparison data in each individual column bearing dissimilar letter (s) have statistically significant difference among them at $p \leq 0.05$. 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)

Table 2. Long-term influence of different organic and chemical fertilizer combinations on the microbial PLFAs contents (µg g^{-1}) in soil.

Treatments	Total PLFAs	Fungal PLFAs	Actinomycetes PLFAs
OM	86.11 ± 6.39ab	10.27 ± 0.82c	9.78 ± 0.95c
HOM	92.52 ± 5.06a	13.24 ± 0.81a	9.41 ± 0.62c
NPK	75.95 ± 5.32bc	9.89 ± 0.63d	9.81 ± 1.12c
NP	71.26 ± 7.81c	11.59 ± 0.46b	9.87 ± 0.26c
NK	66.98 ± 7.34c	11.14 ± 0.86bc	11.42 ± 0.23bc
PK	70.39 ± 3.91c	11.52 ± 0.67 b	11.88 ± 0.70b
Control	67.87 ± 7.71c	0.24 ± 0.69 cd	14.51 ± 1.57a

Treatments comparison data in each individual column bearing dissimilar letter (s) have statistically significant difference among them at $p \leq 0.05$
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)

Table 3. Long-term influence of different organic and chemical fertilizer combinations on the bacterial PLFAs contents (µg g^{-1}) in soil.

Treatments	Bacterial PLFAs	G+ bacterial PLFAs	G− bacterial PLFAs
OM	61.16 ± 4.49b	36.61 ± 2.51a	20.55 ± 1.27b
HOM	70.52 ± 3.63a	33.05 ± 2.04b	23.49 ± 0.86ab
NPK	55.12 ± 3.52c	19.02 ± 1.61c	26.34 ± 1.43a
NP	48.72 ± 6.69cd	15.05 ± 2.82d	22.56 ± 2.49ab
NK	44.52 ± 6.25 d	12.93 ± 1.97d	20.89 ± 2.59b
PK	46.16 ± 2.51 d	14.86 ± 1.01d	20.84 ± 0.65b
Control	41.45 ± 5.09 d	7.40 ± 0.90e	24.14 ± 3.46ab

Treatments comparison data in each individual column bearing dissimilar letter (s) have statistically significant difference among them at $p \leq 0.05$
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)

Discussion

Residual effect of sole and combined application of organic manure (OM) and N, P and K chemical fertilizers is evident from the data on soil nitrogen contents (Table 1). The NO_3^- –N concentration in soil was higher than NH_4^+ –N, being greater under OM and HOM amendments followed by NP and NPK fertilizer treatments. The NH_4^+ –N contents were not matching with the nature of treatments, even though being the highest under OM treatment. Organic manures are known to enhance biological nitrogen fixation by supplying organic carbon required by N-fixing bacteria (Bitew & Alemayehu, 2017). This factor also contributed to total soil nitrogen (TSN) under OM applied treatments over NPK fertilizers. Lower N losses in the manure amended plots due to slow release of N could also be responsible for an increased TSN (Bhandari *et al.,* 2002). Treatments with mineral fertilizers especially combined NPK or NP application enhanced the NO_3^- –N content in soil if compared to control plots; however, PK and NK treatments differed non-significantly from control. Although, chemical fertilizers have limited direct impacts on soil improvement; however, their use can enhance soil biological activity by improving the crop residue return, SOM, soil acidification, and system's productivity (Ge *et al.,* 2008). Organic and conventional fertility management practices have little difference on both diazotrophic and total bacterial communities (Orr *et al.,* 2012). Microbial community structure under the OM and PK applications showed higher richness and diversity, and was significantly different from those of all four N plus treatments (NK, NP, NPK, 1/2 NPK+OM), and CK (Ge *et al.,* 2008). Nevertheless, N fertilizer could be a key factor that counteracts the effects of other mineral fertilizers on soil microbiota. Therefore, shift from yield- to soil-based N management, perfectly executed on a site-specific basis could overturn the current OM decline in arable lands (Khan *et al.,* 2007; Szulc *et al.,* 2018). In line with such findings, integrated use of organic manures and mineral fertilizers is recently being promoted for enhancing cropland productivity, and sequestration of C and N in soil (Yadav *et al.,* 2017).

Kinetics of SOM transformation from OM input to the final formation of an N pool (including mainly amino acids) and CO_2 emission, bestows the principal role to soil organisms included in the entire food-web, where microorganisms are the primary decomposers (Holtkamp *et al.,* 2011; Zanella *et al.,* 2018). 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. Amino acids are the major resource of SON, which is a principal element that regulates the functioning, dynamics and diversity of terrestrial ecosystems (Kögel-Knabner, 2006). Under this long-term study, total amount of amino acids was highest with the full dose of OM being significantly higher than that with HOM (half rate of both OM and NPK fertilizers). It reveals the differential impact of organic manure and chemical N, P and K fertilizers on SOM stabilization in soil. An increase in amino acids content with the application of organic manure was also reported earlier (Kumar *et al.,* 2002; Bibi *et al.,* 2018). High amino acid contents under organic amendment were due to changes in the microbial community structure, and increase in available N and organic C in soil (Stark *et al.,* 2007).

In this study, amino acid contents revealed treatments response similar to that for total microbial PLFAs, bacterial PLFAs and G+ve bacterial PLFAs, as found earlier by Broughton *et al.* (2015). Some other studies also revealed a positive correlation between soil amino acids and microbial N (Geisseler & Horwath, 2008; Hofmockel *et al.,* 2010). Higher contents of amino acids in manure amended soil (OM treatment) were dominantly due to increased proliferation of microbes especially G+ve bacteria. However, supplementation of NPK fertilizer in HOM treatment enhanced the total and bacterial PLFAs, and to some extent the G–ve bacteria. Zhang *et al*. (2014) found that the abundance of fungal PLFAs was lower, while branched PLFAs were significantly higher in the OM treatment than under control and NPK treatments' PLFAs in soil macro-aggregates. Our results also indicate that manure amendment favored proliferation of branched PLFAs especially that of G+ve bacteria.

Conclusions

This study encompasses the long-term impact of organic and inorganic fertilization on nitrogen build-up in wheat and maize cultivated soil. There were significant changes in amino acids and PLFAs concentrations differing with the type of fertilizer treatments, viz., OM, HOM and NPK. Total nitrogen and NO_3^- –N contents in soil increased significantly under manure amendment but not with NPK treatment. Organic manure significantly enhanced G+ve bacteria abundance and amino acids accumulation over other treatments. Contrastingly, NPK treatment significantly improved fungal abundance as against organic manure. Among the mineral nutrients, N and P improved G–ve bacterial PLFAs greatly. An obvious positive impact of K fertilizer was on actinomycetes PLFAs being higher than under OM and other inorganic fertilizer treatments, although actinomycetes PLFAs were at the highest in control. It indicates that application of OM and NPK fertilizers discourage the actinomycetes populations in the soil; however, nitrogen needed for plant growth is enhanced.

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