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Ecological restoration of soil respiration, microbial biomass and enzyme activities through broiler litter application in a calcareous soil cropped with silage maize

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ABSTRACT

A field experiment was conducted to compare the effect of different rates of urea-nitrogen (N) and broiler litter-N on CO_2 production, microbial biomass C (MBC), urease, alkaline phosphatase and invertase activities of a calcareous soil during 100 days of maize growth under field conditions. The experiment consisted of seven N treatments (i.e., control/unfertilized plots and plots received 100, 200, 300 kg N ha^{-1} from urea and broiler litter alone) with four replications. Results showed that urea and broiler litter applications had a significant influence ($P < 0.05$) on all the measured soil biological properties and maize production. However, the effect of N treatments on soil properties much depended upon the time after fertilizer application, the rate of N applied and the type of fertilizer. The averaged CO_2 production (soil respiration) from the surface soil increased with broiler litter and urea additions by 6 and 11% relative to the control soil, respectively. Similarly, urea and broiler litter additions resulted in a significant increase in soil MBC (43–136%), while the soil MBC value with broiler litter treatments was 28% greater than that with urea treatments. Broiler litter and urea treatments had also greater activities of soil urease (0–76%), alkaline phosphatase (9–58%) and invertase (6–31%) than the control treatment. Nonetheless, broiler litter addition had a greater effect on soil alkaline phosphatase (12%) and invertase (17%) than urea addition, whereas urease activity in urea-treated soils was 13% higher than that in broiler litter-treated soils. Most soil biological properties were improved with increasing the level of urea-N and broiler litter-N, but broiler litter applied at 200 and 300 kg N ha^{-1} had the best and comparable effect on soil biological properties. Additionally, urea and broiler litter additions to this calcareous soil resulted in a significant increase in maize growth and production over the unfertilized plots. However, the application of both broiler litter and urea at 200 and 300 kg N ha^{-1} brought about similar maize biomass production, suggesting an adequate maize production could be obtained with urea and broiler litter when both are applied at 200 kg N ha^{-1}. The results of this study also indicated that the soil MBC, CO_2-C production and urease activity were significantly related with maize yield. In brief, broiler litter application can restore soil microbial biomass and activities in the semi-arid areas of Central Iran, where soil organic matter (SOM) level is very low due to low organic C inputs. Broiler litter has a greater effect on soil properties than urea, highlighting the importance of this organic fertilizer in ecological restoration of cropland soils for a sustainable cropping system in the studied area.

1. Introduction

In arid and semi-arid areas, low soil organic matter (SOM) content and water availability are often the main limiting factors for the activity of soil microbial community (Li and Sarah, 2003; Conant et al., 2004; Bastida et al., 2006; Vineela et al., 2008) with a consequence for plant growth and production. The low inputs of organic materials into the surface soil and excessive agricultural practices may have a major influence on soil functioning and quality due to reduced soil carbon (C) contents, nutrient depletion and ultimately soil degradation in arid and semi-arid agroecosystems (Bastida et al., 2006; Raiesi, 2007). Therefore, maintenance of SOM and soil quality is important for the long-term productivity of agroecosystems in arid and semi-arid regions. As a result of low productivity, high inputs of external chemical fertilizers into nutrient deficient arid and semi-arid soils are essential to maintain soil fertility for sustained crop production (Zingore et al., 2008). Chemical nitrogen (N) fertilizers, especially urea, are the major N inputs into

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arid and semi-arid soils for obtaining a satisfactory yield production (Irshad et al., 2002; Zhang et al., 2008; Gheysari et al., 2009). The application of urea and other N fertilizers has, however, been reported to influence most biologically mediated processes in soils (Mahmood et al., 1997; Raiesi, 2004; Akiyama et al., 2004; Yan et al., 2007), which are important in C sequestration and mineralization, and nutrient cycling (Bastida et al., 2006). In arid and semi-arid soils with low SOM levels, an increase in mineral N availability following application of N fertilizers stimulates microbial activity and C mineralization, which would lead to C losses in the long-term (Mahmood et al., 1997; Raiesi, 2004). Excessive application of chemical N fertilizers can also result in N losses via leaching, denitrification and volatilization under specific conditions; leading to environmental pollution (Gioacchini et al., 2002; Akiyama et al., 2004; Zhang et al., 2008).

There are different ecological options for the restoration and maintenance of SOM, soil quality and fertility, among which the application of organic manures could be an effective alternative to maintain an adequate input of organic matter into arid and semi-arid soils (Williams, 1999; Barzegar et al., 2002; Ros et al., 2003; Min et al., 2003; Vineela et al., 2008; Liu et al., 2010). The application of organic manures would ecologically result in more advantages over mineral fertilizers due to improvements in soil ecological processes and indicators such as structure, aggregate stability, soil nutrient exchange capacity, water holding capacity, soil bulk density, microbial biomass and activity, and even crop yields (Haynes and Naidu, 1998; Barzegar et al., 2002; Manna et al., 2007; Verma and Sharma, 2007; Liu et al., 2010). In addition, organic manures increase soil C and N pools by increasing protected SOM within aggregates leading to enhanced C sequestration in soil (Williams, 1999; Sainju et al., 2008; Nyiraneza et al., 2009; Ghosh et al., 2012). The sequestration of organic C in soil can potentially reduce the rate of atmospheric CO_2 enrichment. The positive influence of organic manures on soil fertility and productivity is particularly significant when applying high quality organic amendments (Cordovil et al., 2007; Azeez et al., 2010; Azeez and Averbeke, 2010). The application of organic fertilizers is indeed a desirable practice for ecological restoration of degraded cropland soils by promoting plant growth, conserving microbial community diversity and ecological functions, and ultimately alleviating the constraints to sustainable cropping systems in arid and semi-arid environments (Ros et al., 2003; Abbasi and Khizar, 2012). Certainly, these are in line with the proposed principles of ecological engineering (Mitsch and Jorgensen, 2003) and in the context of soil ecological knowledge for restoration of ecological soil indicators such as microbial activity, biomass and diversity, and enzyme activities (Heneghan et al., 2008).

Soil microbial activity, biomass and enzyme activities play a key role in nutrient cycling, SOM decomposition and other soil functions (Powlson and Jenkinson, 1981; Bastida et al., 2006; Vineela et al., 2008). These soil properties are regularly used as indicators of soil quality and fertility, and have been reported to be greatly and differently affected by organic and inorganic amendments (Parham et al., 2003; Liu et al., 2010). Soil microbial properties are of great importance in determining the sustainability of agricultural management systems, and are becoming increasingly used to evaluate the influence of organic farming practices on soil quality and fertility due to their quick response, high sensitivity, ecological relevance, and ability to present information that integrates many environmental factors (Doran et al., 1996; Azeez et al., 2010; Liu et al., 2010).

Manures and other organic and inorganic amendments can have numerous positive influences on soil microbial and biochemical properties including soil microbial biomass, activity and enzymes (Parham et al., 2003; Cordovil et al., 2007; Vineela et al., 2008; Azeez et al., 2010; Liu et al., 2010). Furthermore, soil microbial biomass and soil enzyme activities respond much more quickly to the inputs of organic and inorganic materials as compared to total soil organic matter (Powlson and Jenkinson, 1981; Doran et al., 1996). Therefore, measurement of microbial biomass and activities provides a sensitive indication of organic matter turnover and nutrient cycling. Most studies have reported that application of organic amendments resulted in greater increases in soil microbial biomass and activities as compared to inorganic fertilizers in arid and semi-arid soils, and in soils from other climates (Mahmood et al., 1997; Mandal et al., 2007; Saha et al., 2008a, 2008b; Garg and Bahl, 2008; Zhao et al., 2009; Abbasi et al., 2010; Vineela et al., 2008; Liu et al., 2010). Mahmood et al. (1997) studied the influence of urea and farmyard manure (FYM) on soil carbon availability and microbial biomass and confirmed that fertilizer application significantly influences the carbon availability as well as the size and activity of microbial biomass. Mandal et al. (2007) also suggested that the microbial biomass and soil enzyme activities were controlled by the long-term manure and fertilizer application. Saha et al. (2008b) studied the long term application of FYM and mineral fertilizers on soil enzyme activities, and their results showed that manure application increased dehydrogenase, acid and alkaline phosphatases, cellulase and protease activities. They concluded that application of FYM directly or indirectly influenced soil enzyme activities. Garg and Bahl (2008) found that among different organic manures, poultry manure recorded the highest alkaline phosphatase followed by FYM, green manure and crop residue. Parham et al. (2003) and Liu et al. (2010) also reported that organic fertilizers enhanced microbial biomass and enzymes activities more than inorganic fertilizers.

Among organic amendments, broiler litter has received considerable attention due to its high C quality, great N concentration and low C/N ratio (Qafoku et al., 2001; Cordovil et al., 2007; López-Mosquera et al., 2008). These factors exert a significant control on soil microbial activities and biochemical reactions (Cordovil et al., 2007). Broiler litter is a rich source of C and nutrients, and provides large quantities of important nutrients for plant growth (Qafoku et al., 2001; Cordovil et al., 2007; López-Mosquera et al., 2008; Singh et al., 2009; Abbasi et al., 2010) and the activity of soil microorganisms (Zhao et al., 2009; Abbasi et al., 2010; Abbasi and Khizar, 2012). Use of broiler litter resulted in the greatest increased maize and corn yield as well as stimulated microbial activities (Abbasi et al., 2010; Abbasi and Khizar, 2012). Although application of inorganic N fertilizers in soils with N limitation increases crop growth, it may initially stimulate soil microbial activities and consequently increases C losses (Raiesi, 2004; Zhao et al., 2009; Abbasi et al., 2010). This is especially important in arid and semi-arid calcareous soils, where SOM content is low due to low C input rates as a result of low net primary production. However, it has been shown that application of broiler litter not only improves crop growth and yield in these soils, it also increases soil C and N contents with a consequence for enhanced soil microbial activities, biomass and enzymes (Paul and Beauchamp, 1996; Vineela et al., 2008; Abbasi et al., 2010; Abbasi and Khizar, 2012; Cordovil et al., 2005). Application of urea alone may promote microbial biomass and activity due to the increase in soil available N content (Cordovil et al., 2005), but lower MBC and activity were also found with urea than broiler litter applications due to a reduction in soil pH following urea application under maize growth (Abbasi et al., 2010).

Although the application of different manures and chemical fertilizers is considered an appropriate tool for improving soil fertility and crop production in calcareous soils of Iran (Hojati and Nourbakhsh, 2006; Gheysari et al., 2009), very few studies have been conducted to evaluate the effect of different rates of N from broiler litter and urea on soil respiration, microbial biomass and

enzyme activities in these arid and semi-arid soils. Broiler litter might be a valuable nutrient source over urea for the maintenance of soil quality and silage maize production in these areas. Over the last two decades, there has been an expansion of poultry production with an increase in broiler manure in Central Iran. Therefore, the primary objective of this study was to investigate and compare the short-term effects of increasing rates of N from urea and broiler litter sources on soil respiration, biomass and enzymes (urease, alkaline phosphatase, and invertase) and irrigated silage maize (*Zea mays* L.) performance in a calcareous soil with low organic matter content (0.62%) under field conditions. The second objective was to test whether the application of broiler litter can be used as a replacement of urea to maintain soil microbial activity and biomass as well as maize production at the levels comparable to or higher than urea application. This could be a promising practice, helping to increase soil physio-chemical and biological properties following C, N and P additions from broiler litter, which are indeed crucial for the ecological restoration of degraded calcareous soils in arid and semi-arid areas.

2. Materials and methods

2.1. Field description

This study was conducted at the Research Station of Shahrekord University (50° 49′ E, 32° 21′ N, 2050 m above sea level). The experimental site was characterized as semi-arid with the mean annual rainfall of 334 mm and annual temperature of 10.8 °C. Soils of the area are calcareous with more than 30% calcium carbonate in the surface layer, which have developed in limestone. The study soil was classified as Clayey Skeletal, Carbonatic, mesic Fluventic Haploxerep. The site has never fertilized with any organic manures and inorganic fertilizers and never cultivated five years before the experiment. Soil samples (0–30 cm depth) were obtained and analyzed for some characteristics before the initiation of the experiment (Table 1). The study soil had 39% clay, 39% silt and 22% sand (clay loam texture) with low organic C (3.6 g kg^{-1}) and total N (0.22 g kg^{-1}) contents (Table 1). Broiler litter was obtained from poultry farms of Shahrekord University. The broiler litter was made up of the poultry manure mixed with different proportions of straw and sawdust as bedding materials. Three broiler litter sub-samples were air-dried and ground to 1 mm for chemical analysis. Electrical conductivity (EC), organic C, total and inorganic N, and P were measured for broiler litter (Table 1). A large area was devoted to silage maize cropping systems in Central Iran. This crop is cultivated under furrow irrigation and is commonly fertilized with chemical fertilizers.

Table 1
Characteristics of the calcareous soil and broiler litter used in this study.

Property	Unit	Soil	Broiler litter (B)
Clay	(g kg^{-1})	390	–
Silt	(g kg^{-1})	390	–
Sand	(g kg^{-1})	220	–
pH	–	8.43	8.21
EC	(dS m^{-1})	0.25	1.14
OC	(g kg^{-1})	3.6	368
CaCO3	(g kg^{-1})	344	–
CEC	(cmol + kg^{-1})	24	–
TN	(g kg^{-1})	0.22	26
NH_4^+-N	(mg kg^{-1})	7.5	20
NO_3^--N	(mg kg^{-1})	1.5	6
Inorganic N	(mg kg^{-1})	9.0	26
P	(mg kg^{-1})	11	7423
C/N	–	16.4	14.15

2.2. Experimental layout

The experiment was arranged as completely randomized blocks using seven N fertilizer treatments with four replications. N treatments (NT) were broiler litter and urea fertilizer each applied at rates of 100, 200, 300 kg N ha^{-1} on a soil dry weight basis and a control (unfertilized) soil cropped with silage maize. The high rate of N was selected because local farmers utilize 250–340 kg N ha^{-1} for conventional maize cropping systems in arid and semi-arid areas of Iran (Gheysari et al., 2009), without considering N losses and its consequence for soil quality. The urea-N was split into three applications during the maize vegetative stage. One-third of the urea-N was applied at planting, one-third as a side-dress application when plants were 2–3 weeks old, and one-third at tasselling. Broiler litter was incorporated into the soil immediately after application and before planting. Phosphorus (P) fertilizer (triple superphosphate) was applied to urea-fertilized plots at a rate equivalent to the total P added by broiler litter.

In total, twenty eight plots (3.6 m × 9 m) were arranged, and six rows with a distance of 60 cm were set up in each plot. The distance between plots and between blocks was 2 m, and for each plot two rows were considered as buffer zone. In each row, three silage maize (Single Cross 704) seeds were sown by hand per each hole at the depth of 5 cm. Maize plants were thinned to one plant per hole at the 3-4th leaf stage after 3 weeks for a plant density of 120,000 plants ha^{-1} (about 390 plants per pot). Irrigation was carried out by furrow method at 6-day intervals, which is common in the study area.

Soil biological parameters including CO_2 (flux) production, microbial biomass C and enzyme activities (urease, alkaline phosphatase and invertase) were monitored during the growing season at different sampling times (i.e., 20, 40, 60 and 80 days after planting maize crop and 20 days after harvesting maize crop) and their responses to urea and broiler litter additions were evaluated. The last sampling time was 20 days after maize harvest and 100 days after fertilizer application. The maize was harvested by cutting shoots at the soil surface for aboveground biomass (AGB) dry weights at day 80. Twenty plants from middle rows were harvested and dry weights of the silage biomass were measured after drying the sub-samples at 80 °C until a constant weight. The maize AGB was expressed as dry matter silage yield (Mg ha^{-1}). Soil biological parameters were determined at 100 days (i.e., 20 days after maize harvest) to assess the effects of fertilizers and post-harvest on soil properties.

2.3. Determination of soil respiration

For measurement of soil respiration (i.e., CO_2 production), three 1.8 L plastic jars with sealed top and open bottom were inserted 3 cm into surface soil in rows for each plot, and left in the soil during the study. A plastic vial containing 20 ml 1 M NaOH was placed inside the jars for CO_2 absorption and collection. The amount of CO_2 evolved from the soil was determined by back-titrating the alkali with 0.25 N HCl after precipitating the carbonate with $BaCl_2$ solution (Alef and Nannipieri, 1995). The CO_2 evolution was expressed as mg CO_2-C m^{-2} soil.

2.4. Determination of microbial biomass and enzyme activities

Five soil samples (50 g) were taken randomly from each row at 25 cm depth by auger, transported to the laboratory and mixed thoroughly to obtain composite samples for each plot and sieved (2 mm mesh size) for determination of soil microbial biomass C (MBC) and enzyme activities. Soil MBC was determined by the chloroform-fumigation incubation method (Alef and Nannipieri,

Table 2
Analysis of variance (ANOVA) results (*F* values) for soil CO_2-C production, microbial biomass carbon (MBC), metabolic quotient (qCO_2); urease, alkaline phosphatase (ALP) and invertase activities and geometric mean of enzyme activities (GME) with two main factors, N treatments and sampling time under maize cropping.

Source of variation	*df*	CO_2-C	MBC	qCO_2	Urease	ALP	Invertase	GME
N treatment (NT)	6	22.5**	215**	9**	24.8**	33**	12.8**	4.0**
Sampling time (ST)	4	6165**	1034**	350**	161**	710**	855**	1935**
NT × ST	24	2.2*	53.3**	11.9**	8.3**	6.8**	9.8**	14.5**
C.V.,%	–	3.5	7.7	15.3	22.2	12.2	11.7	2.45
$LSD_{0.05}$ (for NT)	–	91.1	7.8	11.8	0.197	76	49.1	0.31
$LSD_{0.05}$ (for ST)	–	21.6	4.8	7.7	0.193	36.6	25.5	0.12
$LSD_{0.05}$ (for NT × ST)	–	57.1	12.8	20.0	0.51	96.8	67.4	3.4

* $P < 0.01$.
** $P < 0.001$.

1995). In brief, two 40 g subsamples were put in 100 ml special glass beakers. One beaker was fumigated with ethanol-free chloroform in a vacuum desiccator for 24 h at room temperature. The chloroform vapor in the desiccator was removed by repeated evacuations. All beakers were placed in plastic jars with a vial containing 15 ml of 0.5 M NaOH for CO_2 trap. The jars were closed tightly and incubated for 10 days at 70% of soil water holding capacity and 25 ± 1 °C. The evolved CO_2 was determined as described above. Soil MBC content was calculated as

$$\text{MBC} = \frac{F_c}{k_c}$$

where MBC is soil microbial biomass C (mg kg^{-1} soil) expressed on an oven-dry (105 °C) weight basis, F_c is flash of CO_2 and k_c is the recovery factor equivalent to 0.45 (Jenkinson and Ladd, 1981). The metabolic quotient (qCO_2) was calculated by dividing soil basal respiration from un-fumigated soil by MBC and expressed as μg CO_2-C mg^{-1} MBC day^{-1} for each treatment.

Urease activity was determined by the method of Tabatabai and Bremner (1972) using pure urea as substrate under standard conditions. Alkaline phosphatase activity was measured by the method of Eivazi and Tabatabai (1977) with a modified universal buffer (MUB). For measuring the activity of invertase, saccharose was used as substrate and determined by the method of Schinner and Mersi (1990). Enzyme activities were all expressed on an oven-dry (105 °C) weight basis.

For each N treatment, the geometric mean of enzyme activities (GME) was calculated as:

$$\text{GME} = \sqrt[3]{(\text{UR} + \text{ALP} + \text{INV})}$$

where UR, ALP and INV denote urease, alkaline phosphatase and invertase activities, respectively (Hinojosa et al., 2004; Paz-Ferreiro et al., 2012). The geometric mean is a common index to combine data and information from various soil enzymes with different range of values and units. This index was used as the overall enzyme activities as well as general microbiological activities in polluted soils (Hinojosa et al., 2004) and in the sewage sludge amended soils (Paz-Ferreiro et al., 2012).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to test the impact of N treatments (NT) on maize AGB dry weight using SAS software. Two-way analysis of variance (ANOVA) was performed to check the impact of N treatments on soil biological parameters. Differences amongst means were analyzed for repeated measures in general linear model (GLM) procedures of SAS program, where sampling time was considered as a repeated measure. Differences between treatment means were tested using the Fisher's Protected least significant difference (LSD) post hoc test. Differences were considered significant only when the probability level was lower than 0.05 ($P \leq 0.05$). The significance of relationships between soil variables and maize yield was determined using Pearson's correlation coefficients.

3. Results and discussion

3.1. Soil respiration (CO_2-C production)

Soil respiration is a component of the global C cycle and is considered as one of the major pathways of CO_2 flux in the global C cycle (Conant et al., 2004). A small change in soil respiration could influence the CO_2 annual input to and removal from the atmosphere (Conant et al., 2004). The ANOVA showed significant effects of N treatments (urea and broiler litter) and sampling time on the cumulative soil respiration (Table 2). The N treatments × sampling time interaction was also significant for soil respiration. Results indicate that soil CO_2-C production was similar for fertilized and unfertilized plots on 20-day sampling time, but was greater with the addition of urea and broiler litter as compared to the control treatment on the rest of sampling times (Table 3). This indicates that soil microbial activity as a component of soil respiration may be co-limited by both C and N availability, and that plant growth and root activity (that contribute to soil respiration) may be N limited in the study soil. Increased soil respiration in fertilized plots could be probably due to the addition of organic C, N and P to the soil from broiler litter and to the increased maize growth in both broiler litter- and urea-fertilized plots. Similar results were reported by others for poultry manure (Pengthamkeerati et al., 2005; Abbasi and Khizar, 2012), urea (Raiesi, 2004; Abbasi and Khizar, 2012) and FYM fertilizers (Mahmood et al., 1997). Pengthamkeerati et al. (2005) showed that soil CO_2 efflux was greater in poultry litter treatment as compared to unamended soil, indicating the positive effect of this organic manure on soil CO_2 efflux because of more readily available C and greater nutrients. Abbasi and Khizar (2012) concluded that the increase in CO_2-C evolution in urea-amended compared to unamended Inceptisols with low C (5.9 g kg^{-1}) and N (0.56 g kg^{-1}) may indicate that N was a limiting factor for soil CO_2 production. Mahmood et al. (1997) reported that both urea and FYM fertilizers increased C mineralization in wheat and maize cropping systems.

The greater soil respiration in manured plots relative to urea and control plots could be possibly due to the input of easily mineralizable organic compounds, the availability of C substrate and other essential nutrients (i.e., N and P) for soil microorganisms in broiler litter. This may suggest that soil microbial activity may be limited by N availability. The input of easily mineralizable organic materials and essential nutrients from broiler litter would indeed stimulate microbial and root activities with greater contribution to soil respiration. Poultry litter application enhanced CO_2 efflux in a maize soil amended with poultry litter under field conditions due to the input of easily available C from manure (Pengthamkeerati

Table 3
Cumulative soil CO_2-C evolution, microbial biomass C (MBC) and metabolic quotient (qCO_2) at three rates of urea (U) and broiler litter (B) at five sampling times during maize growth under field conditions. Values are means ($n = 4$) ± SD.

N treatment (NT)	Sampling time (ST) during the maize growing season (day)					
	20	40	60	80	100[a]	Mean (NT)
Cumulative CO_2-C evolution (mg m^{-2})						
B0U0 (C)	363 ± 15.0A	725 ± 60.1B	1082 ± 95.7D	1507 ± 139B	1776 ± 134C	1091C
B100	385 ± 16.6A	772 ± 36.6AB	1146 ± 52.5BC	1588 ± 79.3B	1884 ± 114B	1155ABC
B200	394 ± 17.9A	791 ± 31.9A	1177 ± 34.4AB	1624 ± 67.0A	1913 ± 84.4A	1180ABC
B300	401 ± 14.3A	816 ± 32.6A	1212 ± 39.0A	1658 ± 45.2A	1957 ± 46.5A	1209A
Mean (B)	393 ± 16.3	793 ± 33.7	1178 ± 42.0	1623 ± 63.8	1918 ± 81.6	1181
U100	399 ± 4.4A	766 ± 35.0AB	1106 ± 61.5CD	1525 ± 96.1B	1768 ± 128C	1113BC
U200	397 ± 14.0A	788 ± 28.8A	1158 ± 48.8ABC	1622 ± 82.8A	1914 ± 99.5A	1176ABC
U300	384 ± 31.0A	788 ± 48.0A	1179 ± 77.1AB	1650 ± 103A	1960 ± 114A	1192AB
Mean (U)	393 ± 16.5	781 ± 37.3	1148 ± 62.5	1599 ± 94	1881 ± 114	1160
Mean (ST)	389 ± 15.9e	778 ± 43.7d	1152 ± 66.7c	1596 ± 98.9b	1882 ± 110a	–
Microbial biomass C (MBC) (mg kg^{-1})						
B0U0 (C)	78.3 ± 3.30Fb	145 ± 21.3Ca	58.3 ± 10.0Dc	30.3 ± 5.0Ce	95.1 ± 8.00CDd	71.2F
B100	255 ± 6.4Ba	96.7 ± 3.40Ec	85 ± 6.40Cc	65.0 ± 5.0Bd	115 ± 4.2Ab	123C
B200	263 ± 3.90Ba	167 ± 5.40Bb	85 ± 3.30Cd	74.0 ± 5.0Bd	100 ± 6.8BCDc	138B
B300	295 ± 33.7Aa	182 ± .40Ab	152 ± 3.30Ac	99.7 ± 5.0Ad	110 ± 8.0ABd	168A
Mean (B)	261 ± 14.7	149 ± 5.10	107 ± 4.30	79.6 ± 5.0	108 ± 6.3	143
U100	198 ± 17.5Da	95 ± 10.0Eb	63.3 ± 11.5Dc	65.0 ± 5.0Bc	87.5 ± 4.8Db	102E
U200	137 ± 3.90Eb	150 ± 8.60Ca	100 ± 5.40Bc	69.3 ± 7.1BCd	104 ± 4.8ABCc	112D
U300	227 ± 9.40Ca	122 ± 8.40Dab	98.3 ± 6.40Bc	62.8 ± 4.3Bd	93.8 ± 4.2CDc	121C
Mean (U)	187 ± 10.3	122 ± 9.00	87.2 ± 5.60	65.7 ± 5.5	95.1 ± 4.6	112
Mean (ST)	207 ± 9.40a	136 ± 11.8b	91.7 ± 6.60c	66.5 ± 5.2d	93.5 ± 6.3c	–
Metabolic quotient (qCO_2) (μg CO_2-C mg^{-1} MBC day^{-1})						
B0U0 (C)	28 ± 8CDc	53 ± 10Dc	118 ± 11Cb	179 ± 20ABa	119 ± 25Ab	99A
B100	56 ± 10ABc	89 ± 4Bb	147 ± 8Ba	167 ± 15Ba	36 ± 10CDc	99A
B200	32 ± 3CDc	60 ± 4CDb	130 ± 12BCa	137 ± 20Ca	30 ± 5Dc	78C
B300	45 ± 20BDCc	74 ± 4BCDbc	86 ± 12Db	169 ± 9Ba	41 ± 10CDc	83CB
Mean (B)	50 ± 11	74 ± 4	121 ± 10	158 ± 15	36 ± 8	87
U100	19 ± 4De	115 ± 13Ab	170 ± 40Aa	140 ± 20Cb	67 ± 12Bd	102A
U200	73 ± 13Ac	76 ± 4BCc	123 ± 12Cb	160 ± 30BCa	35 ± 14Cd	93AB
U300	52 ± 1BCd	87 ± 8Bc	118 ± 15Cb	199 ± 30Aa	55 ± 8BCd	102A
Mean (U)	48 ± 5	93 ± 8	137 ± 22	170 ± 30	52 ± 11	99
Mean (ST)	44 ± 8e	79 ± 7c	127 ± 14b	164 ± 21a	55 ± 15d	–

Within a row mean values followed by different lowercase letters are significantly different at $P < 0.05$ among sampling times (ST) by LSD; Within a column mean values followed by different uppercase letters are significantly different at $P < 0.05$ among N treatments (NT) by LSD; B0U0 (C), not-fertilized soil; B100, B200, B300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from broiler litter; respectively; U100, U200, U300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from urea, respectively.

[a] Sampling time (ST) after maize harvest.

et al., 2005). Nevertheless, total soil respiration measured in the current study is the sum of microbial respiration from microbial activity and root respiration from root activity, and the contribution of these two components to total soil respiration cannot be quantified separately. However, maize aboveground biomass (AGB) was the same for both fertilized soils (see "maize production section" below), and thus the root activity was assumed to be identical. In addition, changes in soil moisture conditions and an increase in soil water holding capacity with broiler litter application could have contributed to higher microbial and root activities (Agbede et al., 2010). Yet, soil respiration is largely dependent upon soil temperature, which was similar for all the N treatments, but showed fluctuations during the study. Soil temperature and soil water content were higher in poultry litter-amended soils compared to the control soils, leading to a greater CO_2 flux from the surface soil (Pengthamkeerati et al., 2005).

Results of this study further show that CO_2-C production from fertilized soils was dependent upon the N rate applied initially. Generally, soil respiration in plots that received 200 and 300 kg N from both fertilizers was similar, but greater than plots that received 100 kg N from both urea and broiler litter (Table 3). Total soil CO_2-C production on the last sampling time was greater with the addition of urea (0–10%) and broiler litter (6–10%) as compared to the control treatment. This means broiler litter was more effective than urea fertilizer at similar N rates in the enhancement of CO_2-C production from the soil surface. These results agree with findings of other researchers (Goyal et al., 1999; Min et al., 2003; Canali et al., 2004; Abbasi and Khizar, 2012). For example, Canali et al. (2004) found that C mineralization in organic fertilized soils was significantly higher than mineral fertilized soils. Application of poultry manures significantly increased the CO_2-C evolution compared with urea (Abbasi and Khizar, 2012), indicating that soil microbial activity increased with organic substrates. In a field and laboratory study, surface soil CO_2 efflux was higher at the beginning of the cropping season after poultry litter had been added and decreased with time (Pengthamkeerati et al., 2005).

3.2. Soil microbial biomass carbon (MBC)

N treatment, sampling time and their interactions had a significant effect ($P \leq 0.001$) on soil MBC contents (Table 3). At all the sampling dates, the amount of MBC was greater in fertilized than unfertilized soils, indicating both C and N would limit microbial growth and MBC in these calcareous soils. This is also supported by C and N limitation on soil respiration. The readily metabolizable C and N in broiler litter and probably increasing root biomass and exudates due to greater maize growth could be the responsible factors contributing to the increased microbial biomass. Similarly,

Simek et al. (1999) reported that soil MBC was increased significantly by manure and fertilizer additions to the soil. Our findings are consistent with the increases in soil MBC following the application of poultry manure (Kaur et al., 2005; Abbasi and Khizar, 2012) and urea (Raiesi, 2004; Kaur et al., 2005; Zhao et al., 2009; Liu et al., 2010; Abbasi and Khizar, 2012) in soils with low soil C and N contents. Raiesi (2004) reported increasing rates of urea-N fertilizer resulted in higher MBC levels in calcareous soils of Central Iran. Kanchikerimath and Singh (2001) reported an increase of microbial biomass after application of FYM and mineral N-fertilizer in a semiarid Cambisol. Data shows that soil MBC increased with increasing rates of urea and broiler litter on most of the sampling times. Greater soil MBC could be due to the supply of labile C, N and P by broiler litter; and available N by urea and P by the added P fertilizer for microorganisms. In the current study, urea fertilized plots also received P fertilizer. Urea and broiler litter may also enhance soil MBC by an increase in plant growth and root biomass.

The results of the current study demonstrate that the highest MBC values were observed with broiler litter applied at 300 kg N ha^{-1} (100–295 mg kg^{-1}) during the growing season (i.e., 20 to 80 sampling dates). However, after maize harvest (i.e., on 100-day sampling time), the highest MBC values were seen with broiler litter applied at 100 and 300 kg N ha^{-1} (110–115 mg kg^{-1}) and with urea applied at 200 (104 mg kg^{-1}), while the lowest value was observed with urea applied at 100 (87.5 mg kg^{-1}). In general, both urea-treated and control plots contained similar soil MBC contents, but lower than manured plots after plant harvest (i.e., during the non-growing season). This indicates that the influence of broiler litter on soil MBC is evident after maize harvest, most likely due to its residual effect. Soil MBC level is controlled by the availability and quality of substrate as well as N and P availability (Bohem et al., 2005). Application of broiler litter increases soil C, N and P contents (López-Mosquera et al., 2008; Singh et al., 2009) and would provide more labile substrates favoring the maintenance of a larger soil MBC. Lower MBC values in urea-treated soils were presumably due to lower supply and availability of C. Previous studies (Hojati and Nourbakhsh, 2006; Yan et al., 2007) reported that the application of organic fertilizers increased soil MBC compared to chemical fertilizers in arid- and semi-arid soils, suggesting microbial biomass is C limited in arid environments. Chemical fertilizers may not replenish soil organic C that is essential as microbial substrate, and would decline soil microbial biomass and activity compared with organic fertilizers. Added urea-N could also combine with native soil C, thus reducing C availability to microorganism (Thirukkumaran and Parkinson, 2000). Consequently, this could cause reduced microbial activity and biomass.

Across all N treatments, soil MBC showed some significant variations and tended to decrease from 207 to 66.5 mg kg^{-1} soil during the growing season, but increased to 93.5 mg kg^{-1} after plants were harvested (Table 3). The decrease in soil MBC could result from decreasing root exudates and possibly low root turnover, while the increase in soil MBC could be due to the addition of maize dead roots after plant harvest. This may further demonstrate that the addition of root exudates would be negligible after crop harvest and consequently would not contribute to soil microbial growth. With plant harvest, essential nutrients remaining are mainly assimilated only by soil microorganisms, and the supply of C from decomposing dead roots would contribute to microbial cell growth and biosynthesis. When averaged across sampling times and N rates, soil MBC was increased by 2-fold with broiler litter and 1.6-fold with urea compared to the unfertilized soil. This would show an increase in microbial activity and root respiration due to the additions of both urea and broiler litter during maize growth. Nevertheless, the highest average value across sampling time was seen with broiler litter when applied at 300 kg N ha^{-1} (Table 3). In addition to the supply of C, N and P, broiler litter may stimulate soil MBC via the enhancement of water holding capacity. It has been shown that poultry litter increased soil water content and available water holding capacity (Agbede et al., 2010).

The microbial metabolic quotient (qCO_2) was calculated as the amount of soil basal respiration per unit MBC (μg CO_2-C mg^{-1} MBC day^{-1}) for each N treatment (Table 3). It shows how efficiently the soil microbial biomass assimilates available C for cell biosynthesis and could be used as indicator of the physiological status of soil microbes (Anderson and Domsch, 1990, 1993). Soil qCO_2 is also considered a sensitive indicator for measuring microbiological activity and substrate quality (Anderson and Domsch, 1990; Wardle and Ghani, 1995) and could be affected by changes in the composition of the microbial population, the availability of substrates and abiotic factors (Anderson and Domsch, 1993; Min et al., 2003; Bohem et al., 2005). The effect of N treatments and their interaction with sampling time was statistically significant ($P < 0.001$; Table 2). Initially, qCO_2 values were greater in fertilized (48–50 μg CO_2-C mg^{-1} MBC day^{-1}) than unfertilized soils (28 μg CO_2-C mg^{-1} MBC day^{-1}). However, a reversed trend was found 40 days after application of both fertilizers. At 60-day sampling time the urea-treated plots (137 μg CO_2-C mg^{-1} MBC day^{-1}) showed greater metabolic quotient values than the broiler litter-treated and untreated control plots (118–121 μg CO_2-C mg^{-1} MBC day^{-1}). At 80-day sampling time the broiler litter-treated plots showed lower metabolic quotient values (158 μg CO_2-C mg^{-1} MBC day^{-1}) than the urea-treated (170 μg CO_2-C mg^{-1} MBC day^{-1}) and untreated control plots (179 μg CO_2-C mg^{-1} MBC day^{-1}). However, at the non-growing season sampling, fertilized plots showed almost similar qCO_2 values (36–52 μg CO_2-C mg^{-1} MBC day^{-1}), but significantly lower than the control plots (119 μg CO_2-C mg^{-1} MBC day^{-1}). Similarly, Thirukkumaran and Parkinson (2000) observed a significant increase in soil qCO_2 with urea addition over the control. High metabolic quotients have been reported with N and manure applications (Min et al., 2003; Bohem et al., 2005).

Generally, the increase in qCO_2 values during the growing season was greater (8%) in urea than broiler litter soils. Our findings are supported by the results of Bohem et al. (2005) and Ge et al. (2010) who reported inorganic treatments had the highest qCO_2 values than organic treatments, most likely due to lower C availability. Min et al. (2003) also reported higher qCO_2 values in chemical fertilized soils compared to that in dairy manure-fertilized soil or the control. They suggested that the increases in soil acidity and low availability of labile C with chemical fertilizers could have resulted in stressed conditions for microbial life. This would mean the diversion of energy and substrate use from microbial cell growth to cell survival and maintenance. Broiler litter supplies a large pool of labile C for the synthesis of microbial cells and thus qCO_2 values were smaller in soils amended with broiler litter compared to soils amended with urea and the control. This finding confirms previous observations that qCO_2 values are lower in soils treated with organic manure than with chemical fertilizers (Bohem et al., 2005).

Changes in qCO_2 may also reflect both inputs and outputs of organic C into the soil, and could be used when changes in C availability influence both microbial activity and biomass in arable soils (Anderson and Domsch, 1990, 1993). Another explanation for the difference in qCO_2 values among treatments could be a shift in the microbial population from r strategists toward K strategists, where large amounts of easily decomposable substrates would favor the r strategists (Bohem et al., 2005). Across all N treatments, significant increases ($P < 0.001$) in microbial metabolic quotients were detected during the presence of maize crop, tending to increase from day 20 (44 μg CO_2-C mg^{-1} MBC day^{-1}) to day 80 (164 μg CO_2-C mg^{-1} MBC day^{-1}). However, after maize harvest qCO_2 values

Table 4
Soil enzyme activities at three rates of urea (U) and broiler litter (B) at five sampling times during maize growth under field conditions. Values are means ($n = 4$) ± SD.

N treatment (NT)	Sampling time (ST) during the maize growing season (day)					
	20	40	60	80	100[a]	Mean (NT)
Urease (μg NH_4-N g^{-1} $2h^{-1}$)						
B0U0 (C)	1.4 ± 0.36Cb	0.89 ± 0.06Dbc	0.4 ± 0.13CDc	0.67 ± 0.20BCc	2.9 ± 0.04ABa	1.3D
B100	1.1 ± 0.23Cb	1.0 ± 0.02CDb	0.96 ± 0.19Bb	0.81 ± 0.33ABb	2.6 ± 0.60Ba	1.3D
B200	1.6 ± 0.27BCb	1.5 ± 0.36BCb	0.78 ± 0.10BCc	0.26 ± 0.10Cd	2.5 ± 0.15Ba	1.3D
B300	3.1 ± 0.85Aa	2.1 ± 0.46ABb	1.8 ± 0.46Abc	1.3 ± 0.40Ac	3.2 ± 0.45Aa	2.3A
Mean (B)	1.9 ± 0.45	1.5 ± 0.28	1.2 ± 0.25	0.79 ± 0.28	2.8 ± 0.4	1.6
U100	2.0 ± 0.50Bb	1.2 ± 0.20CDc	1.9 ± 0.03Ab	0.26 ± 0.08Cd	3.1 ± 0.24Aa	1.7C
U200	3.0 ± 0.29Aa	1.3 ± 0.09CDb	0.16 ± 0.04Dd	0.39 ± 0.10BCc	3.1 ± 0.60Aa	1.6C
U300	3.3 ± 0.63Aa	2.1 ± 0.35Ab	2.1 ± 0.36Ab	0.45 ± 0.12BCc	2.3 ± 0.51Bb	2.1B
Mean (U)	2.8 ± 0.47	1.5 ± 0.21	1.4 ± 0.22	0.37 ± 0.10	2.8 ± 1.9	1.8
Mean (ST)	2.2 ± 0.45b	1.4 ± 0.22c	1.15 ± 0.19d	0.59 ± 0.19e	2.8 ± 0.37a	–
Alkaline phosphatase (μg PNP g^{-1} h^{-1})						
B0U0 (C)	626 ± 40.8Bb	997 ± 52.5Ca	273 ± 5.3Cc	265 ± 30Cc	201 ± 15.6Bc	473C
B100	625 ± 81.1Bb	1051 ± 200BCa	323 ± 10.2ABCc	531 ± 116Bb	226 ± 16.3Bd	551B
B200	717 ± 51.4Ab	1029 ± 17.6BCa	410 ± 13.6Ad	512 ± 52Bc	266 ± 54.7Be	587B
B300	754 ± 93.4Ab	1504 ± 146Aa	384 ± 11.7ABc	656 ± 21.8Ab	440 ± 77.1Ac	748A
Mean (B)	699 ± 75.3	1195 ± 121	372 ± 11.8	566 ± 63.3	311 ± 49	529
U100	757 ± 149Ab	975 ± 182Ca	303 ± 52.5BCcd	335 ± 15.7Cc	212 ± 12.5Bd	516BC
U200	776 ± 120Ab	1125 ± 120Ba	303 ± 63.8BCc	243 ± 25.7Ccd	200 ± 6.5Bd	529BC
U300	701 ± 105ABb	1041 ± 9.3BCa	329 ± 53.8ABCc	322 ± 46.2Cc	282 ± 17.5Bc	535BC
Mean (U)	745 ± 125	1047 ± 104	311 ± 56.7	300 ± 29.2	231 ± 12.2	527
Mean (ST)	708 ± 91.5b	1103 ± 104a	332 ± 30.1d	409 ± 43.9c	261 ± 28.6e	–
Invertase (μg glucose g^{-1} 24 h^{-1})						
B0U0 (C)	663 ± 36.1BCa	468 ± 21.8Db	519 ± 52.9BCb	53.7 ± 1.9Bd	145 ± 9.6Bc	370C
B100	626 ± 73.5Cb	770 ± 60.3Aa	527 ± 19.7BCc	67.3 ± 14.5Be	152 ± 7.9Bd	428B
B200	608 ± 82.4Ca	597 ± 4.9Cab	533 ± 27.2BCb	36.4 ± 10.9Bd	168 ± 2.6ABc	388BC
B300	714 ± 139ABb	814 ± 88.8Aa	535 ± 21.3Bc	142 ± 6.20Ae	224 ± 4.6Ad	486A
Mean (B)	649 ± 98.3	727 ± 51	532 ± 22.8	81.9 ± 10.5	181 ± 5	434
U100	745 ± 20Aa	567 ± 77.3Cb	465 ± 13.2Cc	31.7 ± 7.4Be	154 ± 11.2Bd	393BC
U200	501 ± 131Db	628 ± 60.1Ca	640 ± 17.7Aa	65.8 ± 6.3Bd	195 ± 14.6ABc	406BC
U300	477 ± 74.2Db	604 ± 104Ca	569 ± 83.2Ba	87.1 ± 15.2ABd	230 ± 15.1Ac	393BC
Mean (U)	574 ± 75.1	600 ± 80.5	558 ± 38	61.5 ± 9.6	193 ± 13.6	397
Mean (ST)	619 ± 79.5a	636 ± 59.6a	541 ± 33.6b	69 ± 8.9d	181 ± 9.4c	–

Within a row mean values followed by different lowercase letters are significantly different at $P < 0.05$ among sampling times (ST) by LSD; Within a column mean values followed by different uppercase letters are significantly different at $P < 0.05$ among N treatments (NT) by LSD; B0U0 (C), not-fertilized soil; B100, B200, B300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from broiler litter; respectively; U100, U200, U300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from urea, respectively.

[a] Sampling time (ST) after maize harvest.

were decreased to 55 μg CO_2-C mg^{-1} MBC day^{-1}, still greater than the initial value (44 μg CO_2-C mg^{-1} MBC day^{-1}) at 20-day sampling time (Table 3). The increase in microbial metabolic quotient was mainly due to the decrease in soil MBC (Table 3). The variations in qCO_2 during the experiment could also be attributed to changes in substrate quality and availability, and microbial population.

3.3. Soil enzyme activities

The activities of soil enzymes were significantly greater under fertilizer application as compared to the unfertilized control on most of the sampling times (Table 4). These findings are consistent with the increases in enzyme activities observed due to the application of poultry manure (Acosta-Martínez and Harmel, 2006; Garg and Bahl, 2008) and urea (Zhao et al., 2009; Liu et al., 2010). However, their responses to N treatments varied with type of N fertilizer, application rates and sampling time. Ros et al. (2003) reported differential influence of various organic amendments on enzyme activities in semi-arid soils. Averaged across the sampling times, application of broiler litter at 100, 200 and 300 kg N ha^{-1} increased the urease activity by 2.2%, 5.2% and 84% over the control, respectively, whilst addition of the same amounts of N from urea increased the urease activity by 35%, 25% and 64% compared to the control treatment. However, the greatest urease activity (involved in N cycling) was observed at the highest rate of broiler litter (2.3 μg NH_4-N g^{-1} soil 2 h^{-1}) followed by the highest rate of urea (2.1 μg NH_4-N g^{-1} soil 2 h^{-1}). Kanchikerimath and Singh (2001) reported that addition of different levels of manures and NPK fertilizer increased the urease activity. Wang et al. (2008) observed that with increasing rates of urea-N urease activity increased relative to the control, because urea is the main and important substrate for this enzyme.

As with soil MBC, urease activity decreased from 2.2 to 0.59 μg NH_4-N g^{-1} soil 2 h^{-1} during the growing season, but tended to increase to 2.8 μg NH_4-N g^{-1} soil 2 h^{-1} after maize harvest across all N treatments (Table 4). This suggests that the contribution of maize to soil urease activity was consistently decreased over the growing season, and that the main origin of soil urease was from microbial sources. Although plants are one source of urease in soil, a decline in root exudates would depress microbial activity and biomass, resulting in decreased urease activity. Additionally, the post-harvest increase in urease activity may be due to the increase in the population of decomposer microorganisms during fine root decomposition. We found a significant and positive correlation between soil MBC and urease activity ($r = 0.57$; $P < 0.01$), showing that soils with high MBC are associated with high urease activity (Table 5).

Similarly, alkaline phosphatase activity (involved in P cycling) increased with increasing application rates of broiler litter and was generally highest at the 300 kg N ha^{-1} rate at different sampling

Table 5
Pearson's correlation coefficients (r) between different soil properties and maize aboveground biomass (AGB) across all urea- and broiler litter-amended soils ($n = 28$).

	AGB	CO_2-C	Urease	ALP	Invertase	MBC	qCO_2	GME
AGB	1.00							
CO_2-C	0.64**	1.00						
Urease	0.56**	0.44*	1.00					
ALP	0.28n.s	0.37n.s	0.60**	1.00				
Invertase	0.35n.s	0.30n.s	0.46*	0. 70***	1.00			
MBC	0.55**	0.50**	0.57**	0.82***	0.64***	1.00		
qCO_2	−0.15n.s	−0.07n.s	0.08n.s	−0.30n.s	−0.37n.s	−0.53**	1.00	
GME	0.39*	0.42*	0.57**	0.96***	0.83***	0.87***	−0.38*	1.00

n.s, not significant, GME, geometric mean for enzyme activities.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

times. In contrast, the activity of this enzyme did not consistently increase with increasing application rates of urea (Table 4). The observed increase in alkaline phosphatase activity as a result of broiler litter addition could be related to higher organic P in this amendment (Table 1), since organic P is the main substrate for the activity of this enzyme. The broiler litter used in this experiment contained 1.7% total phosphorus; hence addition of organic phosphorus to soil following application of broiler litter may have increased soil catalytic activity. Our results are consistent with Garg and Bahl (2008) who showed a significant increase in alkaline phosphatase activity with the application of poultry manure. The increased alkaline phosphatase activity was attributed to high inorganic P in the manure, and was related to P availability. Application of organic manures and wastes may increase SOM contents (Sainju et al., 2008; Zhao et al., 2009; Abbasi et al., 2010; Ghosh et al., 2012), which also played an important role in protecting soil enzymes since they could be immobilized by clay and humus complexes (Pascual et al., 2002; Benitez et al., 2005). The activity of alkaline phosphatase was related to soil organic C contents in fertilized soils (Kanchikerimath and Singh, 2001; Goyal et al., 1999; Zhao et al., 2009). Some researchers also reported that the highest and lowest activity of alkaline phosphatase was found in FYM manure and control treatment, respectively (Bohem et al., 2005). Application of organic fertilizer increased alkaline phosphatase activity compared to control and chemical fertilizer treatments in arid and semi-arid soils (Hojati and Nourbakhsh, 2006).

Increase in alkaline phosphatase activity with broiler litter might be due to the addition of easily decomposable compounds and increased dissolved organic C (DOC) and P (DOP) with a consequence for stimulated microbial activity and biomass (Garg and Bahl, 2008). There were a significant and positive correlation between soil MBC and alkaline phosphatase activity ($r = 0.82$; $P < 0.001$, Table 5); implying soils with high MBC are associated with high activity of alkaline phosphatase. In addition, broiler litter application can increase the lability of native soil P with a consequence for stimulating alkaline phosphatase activity (Waldrip et al., 2011). The activity of alkaline phosphatase is also related to soil pH, with higher activities following manure applications due to an increase in soil pH (Acosta-Martínez and Harmel, 2006). The activity of alkaline phosphatase fluctuated considerably over the different sampling times and N treatments, without a consistent trend. The highest and lowest activities of alkaline phosphatase were observed at 40-day sampling time during maize growth and at 100-day sampling time after maize harvest, respectively. This fluctuation could have resulted from changes in plant physiology and root exudates as well as altered soil and litter organic phosphorus chemistry. Furthermore, the lower activity of alkaline phosphatase during the growing season than non-growing season may indicate that maize plant would contribute to alkaline phosphatase production and activity. In soils with low available P content, both plants and soil microbes contribute to the production of soil phosphatases to meet plant demand for available P.

As with alkaline phosphatase, soil invertase activity in fertilized plots was greater than that in the control plots (Table 4). The average increase was greater with broiler litter (17%) than urea fertilizers (7%) across the N rates. Similar results were reported by previous authors, who observed higher invertase activity in soils treated with broiler litter (Saha et al., 2008b) and other organic fertilizers (Ge et al., 2010; Zhao et al., 2009). Apparently, broiler litter contains a large quantity of soluble carbohydrates, which can stimulate microbial activity, biomass and invertase synthesis. Soil MBC correlated significantly with invertase activity ($r = 0.64$; $P < 0.001$, Table 5), suggesting that an increase in soil MBC with fertilizer application would amplify the activity of soil invertase (Alef and Nannipieri, 1995). This enzyme catalyzes the hydrolysis of sucrose to glucose and fructose, and is widely distributed in soils (Schinner and Mersi, 1990). Invertase is mainly involved in C cycling, with a linkage to the soil microbial biomass (Alef and Nannipieri, 1995). The greatest invertase activity was seen at 20- and 40-day sampling times (Table 4). Urea application at 100 kg N ha^{-1} showed the highest invertase activity than the rest of N treatment at 20-day sampling time. When averaged across sampling time, broiler litter at 300 kg N ha^{-1} had the greatest invertase activity compared with the rest of N treatments, and differences in enzyme activity within urea treatments was non-significant. Invertase activity did not show a consistent trend over the growing season, with no change during the 20 and 40 days, decreases from day 40 to 80 and increases after maize harvest.

The geometric mean of enzyme activities (GME) was calculated to integrate soil enzyme activities to a single value (García-Ruiz et al., 2008). The GME values were similar for fertilized and unfertilized plots at all the sampling times. However, when averaged over the sampling times, GME values for fertilized plots (9.3–10.7) were greater than the control plots (9.1). In addition, organic-treated soils (10) had greater GME values than urea-treated soils (10 vs. 9.4). The highest value (10.7) was found with broiler litter applied at 300 kg N ha^{-1}. Therefore, the GME proved to be a suitable indicator for estimating the response of several enzyme activities to application of organic and inorganic fertilizers (Table 6).

3.4. Maize production

Maize AGB dry weight (stems + leaves) was significantly greater in fertilized soils than in the control soils, but was the same with broiler litter and urea treatments across the N rates (Fig. 1). This shows that maize production is severely reduced without N fertilization at the study site and that the response to the addition of urea and broiler litter fertilizers was almost equal. On average, the amount of silage produced by maize was 24.7 t ha^{-1} in urea-treated soil and 23.9 t ha^{-1} in broiler litter-treated soils, indicating

Table 6
Geometric mean for enzyme activities (GME) at three rates of urea (U) and broiler litter (B) at five sampling times during maize growth under field conditions. Values are means ($n = 4$) ± SD.

N treatment (NT)	Sampling time (ST) during the maize growing season (day)					
	20	40	60	80	100[a]	Mean (NT)
B0U0 (C)	10.9 ± 0.2Aa	11.4 ± 0.2Aa	9.30 ± 0.2Aab	6.80 ± 0.2Abc	7.00 ± 0.2Ab	9.08 ± 0.2D
B100	10.8 ± 0.4Aab	12.2 ± 0.6Aa	9.50 ± 0.1Aabc	8.40 ± 0.5Abc	7.20 ± 0.1Ac	9.62 ± 0.3B
B200	11.0 ± 0.2Aa	11.8 ± 0.1Aa	9.80 ± 0.1Aabc	8.10 ± 0.3Abc	7.60 ± 0.3Ac	9.65 ± 0.2B
B300	11.4 ± 0.1Aab	13.2 ± 0.4Aa	9.70 ± 0.1Ab	8.70 ± 0.3Ab	8.70 ± 0.3Ab	10.7 ± 0.2A
Mean (B)	11.1 ± 0.2	12.4 ± 0.4	9.7 ± 0.1	8.6 ± 0.3	7.8 ± 0.2	10.0 ± 0.2
U100	11.0 ± 0.4Aa	11.5 ± 0.6Aa	9.20 ± 0.2Ab	7.20 ± 0.1Ab	7.20 ± 0.1Ab	9.25 ± 0.3DC
U200	10.9 ± 0.4Aa	12.1 ± 0.3Aa	9.70 ± 0.3Aab	6.8 ± 0.2Ab	7.40 ± 0.1Ab	9.37 ± 0.3BDC
U300	10.6 ± 0.5Aab	11.8 ± 0.3Aa	9.60 ± 0.5Aab	7.40 ± 0.3Ab	8.00 ± 0.1Ab	9.49 ± 0.3BC
Mean (U)	10.8 ± 0.4A	11.8 ± 0.4	9.5 ± 0.3	7.1 ± 0.2	7.5 ± 0.1	9.34 ± 0.3
Mean (ST)	11.0 ± 0.2b	12.0 ± 0.3a	9.6 ± 0.3c	7.7 ± 0.2d	7.6 ± 0.2e	9.58 ± 0.2

Within a row mean values followed by different lowercase letters are significantly different at $P < 0.05$ among sampling times (ST) by LSD; Within a column mean values followed by different uppercase letters are significantly different at $P < 0.05$ among N treatments (NT) by LSD; B0U0 (C), not-fertilized soil; B100, B200, B300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from broiler litter; respectively; U100, U200, U300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from urea, respectively.

[a] Sampling time (ST) after maize harvest.

approximately 43 and 38% higher than that obtained in the unfertilized soils (17.3 t ha^{-1}), respectively. This reveals that the effects of mineral N from urea source on maize growth should be the same as that of organic N from broiler litter source. Similar results were reported by Abbasi et al. (2010) who showed N fertilization from broiler litter and urea increased maize dry matter and grain yields over the unfertilized control soils from Pakistan, with a similar increase for both poultry manure and urea fertilizers. However, the observed AGB values are higher than those values reported by Gheysari et al. (2009), who obtained 19.9 t ha^{-1} of aboveground biomass for silage at 200 kg N ha^{-1} in Varamin region with arid and semi-arid climate.

The higher silage yield in urea-treated maize than in the control maize indicates that urea-N and added mineral P were clearly more readily available to this crop than the mineral N and P released during decomposition of native SOM. This is also confirmed with the observation that higher soil total N (and available P) concentration was detected in urea-fertilized than unfertilized soils at post-harvest sampling (data not shown). The combination of mineral P fertilizer with urea in this study has resulted in a significant increase in soil P availability. The positive yield response to broiler litter is in part due to high N and P contents in this manure (Table 1). In other words, the release of inorganic N and P from broiler litter should have contributed to maize growth and therefore could be important for maize performance in these calcareous soils. We observed greater soil total N and available P concentrations in broiler litter-amended soils than the control soil after maize harvest (data not shown). The application of poultry manure increased total N and available P compared with the control (Singh et al., 2009; Abbasi et al., 2010). Previous studies have shown that 15–74% of the initial organic N in different poultry manures was mineralized within 42 (Gilmour et al., 2004) and 112–120 (Qafoku et al., 2001; Preusch et al., 2002) days of soil incubation. Singh et al. (2009) reported that about 46% of the poultry litter N was mineralized after 60 days of laboratory incubation and that the release of P from poultry litter accounted for 15–17% of the total P. Canali et al. (2004) reported that potential N mineralization was higher in poultry manure amended soils than in urea-fertilized soils over a 6-year field experiment.

Our results show that the greatest maize production was with urea application at 300 kg N ha^{-1} (28.7 t ha^{-1}) and the lowest with broiler litter applied at 100 kg N ha^{-1} (20.6 t ha^{-1}), followed by urea application at both 200 and 300 kg N ha^{-1} (22.7 t ha^{-1}, Fig. 1). Additionally, an increase in maize biomass was associated with

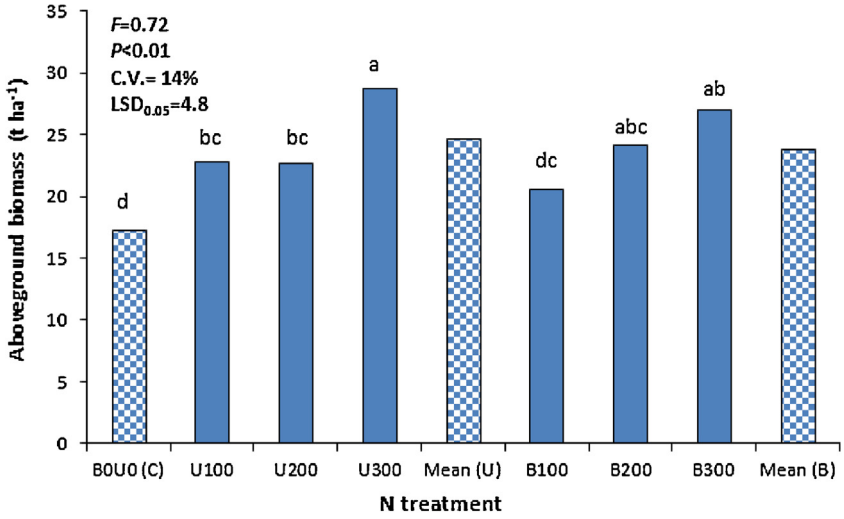

Fig. 1. Maize aboveground biomass (AGB) dry weight (t ha^{-1}) at three rates of urea (U) and broiler litter (B) under field conditions. Values are means ($n = 4$). Values followed by different letters are significantly different at $P < 0.05$ among N treatments (NT) by LSD; B0U0 (C), not-fertilized soil; B100, B200, B300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from broiler litter; respectively; U100, U200, U300 soils fertilized with 100, 200 and 300 kg N ha^{-1} from urea, respectively.

increasing N rates from both urea and broiler litter, and that broiler litter had equal AGB dry weights as compared to urea fertilizer with similar application of N rates. When broiler litter was applied at 300 kg N ha^{-1}, maize biomass was not different between broiler litter applied at this N rate (24.1 t ha^{-1}) and that applied at 200 kg N ha^{-1} (20.6 t ha^{-1}). In contrast, maize biomass was statistically greater with urea applied at 300 kg N ha^{-1} (28.7 t ha^{-1}) than at 200 kg N ha^{-1} (22.7 t ha^{-1}). On the whole, the applications of both urea and broiler litter at 200 and 300 kg N ha^{-1} rates resulted in more or less identical maize production. This means that application of both urea and broiler litter at 200 kg N ha^{-1} is adequate to achieve a satisfactory silage maize production in the study area. This indicates that broiler litter and urea application rates should be based the N requirement of the maize crop, since higher application rates of both fertilizers can contribute to the N and P losses via leaching, volatilization and de-nitrification (Gioacchini et al., 2002; Akiyama et al., 2004; Zhang et al., 2008). Likewise, Gheysari et al. (2009) recommended that application of 225 kg N ha^{-1} resulted in the highest amount of aboveground biomass for silage in other arid and semi-arid areas.

Furthermore, our data shows that the amount of broiler litter application should be adjusted based on its N concentration and availability during the growing season for sustainable maize production. Broiler litter can be used to increase soil C and N contents, and water holding capacity (Agbede et al., 2010; Abbasi and Khizar, 2012), which can increase soil microbial and biochemical properties with a positive influence on maize productivity in semi-arid soils with low SOM contents.

3.5. Correlations between maize biomass and soil parameters

Coefficients of Pearson's correlations between the soil properties and maize aboveground biomass (AGB) are reported in Table 5. Maize AGB dry weights were positively correlated with the CO_2 production ($r = 0.64$, $P < 0.01$), MBC ($r = 0.55$, $P < 0.01$), urease activity ($r = 0.56$, $P < 0.01$) and GME values ($r = 0.39$, $P < 0.05$) averaged all over N treatments. This indicates that the increase in maize AGB following the application of urea and broiler litter is associated with enhanced microbial biomass and activities. It is possible that the addition of poultry and urea fertilizers may also have stimulated maize performance. A strong correlation between urease activity and maize AGB may be due to supplying inorganic N essential for plant uptake through enzymatic processes. However, there was no significant correlation between maize AGB and alkaline phosphatase activity, suggesting microbial transformation of organic P could not be accounted for P uptake by growing maize. In contrast, Zhao et al. (2009) did not observe a significant correlation between crops yield and soil properties in a calcareous soil amended with organic and inorganic fertilizers under wheat–maize cropping systems.

Positive relationships were also observed between CO_2 production and microbial biomass ($r = 0.50$, $P < 0.01$), and urease activity ($r = 0.44$, $P < 0.05$). Moreover, microbial biomass was significantly correlated with the activity of soil enzymes ($r = 0.60$–0.80), suggesting greater enzyme activities with an increase in soil MBC. Similar correlations between soil enzyme activities and MBC contents were reported for calcareous soils (Zhao et al., 2009). The positive correlations between soil enzymes and MBC may also show that soil microbial activity and biomass are an important source of enzymes involved in C, N and P cycling (Hojati and Nourbakhsh, 2006). Soil GME values were positively correlated with CO_2 production, MBC, enzyme activities, but negatively correlated with qCO_2 values ($r = -0.38$, $P < 0.05$). This shows that the GME could be a reliable indicator for assessing soil quality in relation to application of inorganic and organic fertilizers, due to its association with soil respiration and biomass. Relationships between soil properties and soil enzymes could be attributed to improvements in soil physio-chemical properties after additions of organic and mineral fertilizers (Zhao et al., 2009).

There was a strong negative correlation between qCO_2 and MBC ($r = -0.53$, $P < 0.01$). This corresponds well with results from other studies (Wardle and Ghani, 1995; Zhao et al., 2009). However, increased qCO_2 values may indicate an increase in soil microbial activity, which was not observed in this study (Table 5). Overall, the current study shows that broiler litter application results in comparable increases in maize growth and production to urea fertilizer but also stimulates the microbial activity in the soil. However, the large increase in soil microbial activity after the addition of broiler litter did not lead to any greater benefit to plant growth relative to the urea fertilizer.

4. Summary and concluding remarks

The current results showed that additions of urea and broiler litter to the calcareous soils in this study increased soil respiration (CO_2-C production), MBC, urease, alkaline phosphatase and invertase activities, but decreased the metabolic quotient as compared to the unfertilized control. It was further evident that soil MBC was more responsive to urea and broiler litter applications than soil respiration and enzyme activities. Furthermore, the positive effect of broiler litter on CO_2-C production, MBC, alkaline phosphatase and invertase activities was more pronounced than the effect of urea. However, urease activity responded more to urea than broiler litter. Although, broiler litter application could increase SOM, microbial activity and biomass with a better potential for greater enzyme production and activity, the microbial metabolic quotient and urease activity in urea-treated soils were higher than those in soils treated with broiler litter. Generally, with increasing the rates of urea and broiler litter, most of the soil biological properties were improved to a large extent, and broiler litter at 300 kg N ha^{-1} had the greatest effect on these soil properties.

A satisfactory maize production could be obtained with urea and broiler litter when both of them were applied at 200 kg N ha^{-1}. This was due to enhanced soil microbial activity and biomass content. In conclusion, application of broiler litter and urea fertilizers increases the activity and biomass of soil microflora over the growing season, leading to improved silage maize yield in arid- and semi-arid soils of Central Iran. This is particularly more significant with broiler litter application equivalent to 200 kg N ha^{-1} when considering both soil biochemical properties and maize performance simultaneously. In addition, organic N and P from broiler litter resulted in similar maize performance to inorganic N and P from chemical fertilizers.

In brief, the results of the current study indicated that urea fertilizer and broiler litter increased biological activity in this calcareous soil with low organic C and N contents. Nevertheless, broiler litter had a promising effect on most measured soil properties as compared to urea, underscoring the importance of broiler litter in ecological restoration of degraded cropland soils.

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