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Effects of elemental sulfur and soil compaction on microbial biomass carbon and soil enzyme activities

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Abstract

The soil enzymes and microbial biomass play a key role in nutrient cycling and sensitivity to management and environmental change compared to most physical and chemical properties. Thus these parameters have been monitored as essential indicators of soil quality. The aim of this study was to investigate the effects of sulfur and soil compaction on microbial biomass and enzyme activities. Therefore a greenhouse experiment was designed at four levels of sulfur (0, 500, 1000 and 2000kgha^{-1}) and three levels of compaction (C_0, C_1, C_2). Microbial biomass carbon, urease and arylsulphatase activities were determined. The results showed that application of different levels of sulfure dicreased microbial biomass carbon but urease and arylsulphatase activities dicreased at the highest levels of sulfur. Microbial biomass carbon and arylsulphatase activitiy were not influenced by soil compaction but the highest of soil compaction dicreased urease activity.

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Introduction

Soil quality has been defined as "the capacity of a soil to function within ecosystem boundaries to maintain bio-logical productivity, sustain environmental quality, and promote plant health (Doran and Parkin, 1994). Physical and chemical properties have been extensively used to measure soil quality (Parr and Papendick, 1997). However, these properties usually change on a time scale (decades) which is too long for management purposes. In contrast, soil properties based on biological and biochemical activities, such as soil enzymes and microbial biomass carbon(MBC), have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality (Pascual *et al.*, 2000). Soil enzyme activities have been suggested as suitable indicators of soil quality because of their intimate relationship with soil biology, ease of measurement, and rapid response to change in soil management (Dick *et al.*,1996; Riffaldi *et al.*, 2002; Miralles *et al.*, 2007).

Enzymes are mainly from soil microorganisms, and despite their relatively low amounts, play a crucial role in keeping nutrient cycling in soils such as C, N, P, and S (Doran and Parkin 1996; Aon *et al.*, 2001). Soil microbial biomass is intimately linked to nutrient transformations in soil, acting as both a sink and a source of nutrients (Jenkinson and Ladd, 1981; Singh *et al.*, 1989). The changes in soil microbial biomass are important in controlling the turnover of carbon (C) and associated nutrients (e.g. nitrogen (N), phosphorus (P) and sulfur (S)), which in turn regulate nutrient availability for plant uptake (He *et al.*, 1997; Chen *et al.*, 2003).

Soil biological activity mainly including soil enzymatic activity, basal respiration and microbial biomass has been shown to be closely related to both various soil factors including pH values, soil organic matter, soil texture, and modifying factors such as climate, soil moisture and soil temperature regimes, soil agricultural practices and crop rotation (Emmerling *et al.*, 2001; Bending *et al.*, 2004; Bastida *et al.*, 2008). The objective of this study was to investigate the impact of sulfure and soil compaction on soil microbial biomass C and the activities of urease and arylsulphatas under greenhous condition.

Materials and methods

Soils preparation and Soil physical and chemical properties

The three soils were taken from the Farse, Khorasan and Kermanshah provinces. The soil samples sieved and Soil chemical and physical properties were measured before treatmen. Nitrogen was measured using Kjeldahl method (Nelson and Sommers, 1973). Available phosphorous was determined by sodium bicarbonate extraction (Olsen, 1954). Available potassium was measured using flame photometer (emission spectrophotometry) (Knudsen *et al.*, 1982). Iron, manganese, zinc and copper were determined by diethyenetriaminepentaacetic acid (DTPA) method (Baker and Amachar, 1982) using atomic absorption spectrometer (Model PerkinElmer 3110). Acidity of a saturated paste and electrical conductivity of a saturated extract (Rhoades, 1982) were also measured. Organic carbon was measured using wet oxidation (Nelson and Sommers, 1982). The soil texture was determined by the hydrometric method (Gee and Bauder, 1986). Soil moisture at field capacity (_0.033 atm) (Rhoades, 1982) were determined using pressure plates.

experimental design , plant culture and soil sampling

The greenhouse experiment was a randomized complete block design with two factors and four replications. The two factors used were soil compaction and sulfur whit Thiobacillus. Treatments included three level of soil compaction (C_0=non compacted, C_1= C_0+%5C_0, C_2= C_0+%10C_0) and foure level of sulfur within Thiobacillus (S_0=0, S_1=167, S_2=334, S_3=668mgkg^{-1}). Compaction levels were imposed using 2 kg weights, with a little less diameter than the pots diameter according method of Barzegar *et al*(2000). Sulfur and Thiobacillus added to soil according treatments. The seven seeds of corn (cv. 704) planted in each pot and were thinned to four plant after germination. The seedlings were irrigated with water, maintaining the soil moisture content at

field capacity. After 3 months the plants were harvested. The soil were mixed and soil samples were prepared for chemical and microbial properties.

Determination of soil microbial biomass carbon

Microbial biomass carbon (MBC) was estimated following the fumigation extraction (FE) method (Vance *et al*., 1987). Two portions of moist soil (25 g oven-dry soil) were weighted. The first one (non fumigated) was extracted with 100 ml of 0.5 M K_2SO_4 for 30 min by oscillating shaking at 200 rpm and filtered (Whatman no. 42), the second one was fumigated for 24h at 25° C with ethanol-free $CHCl_3$. Following fumigant removal the soil was extracted similarly for the non fumigated one. Organic C in the extracts was determined after oxidation with potassium dichromate at100° C for 30min and microbial C was calculated as follows:

microbial biomass C = EC:k_{EC} where EC is the difference between organic C extracted from fumigated soils and organic C extracted from non-fumigated soils and k_{EC} = 0.38 (or 1/2.64) (Vance *et al*., 1987).

Determination of soil enzymatic activity

Urease activity was determined by the method described by Kandeler and Gerber (1988). five grams of moist soil, was placed into a 100-mL Erlenmeyer flask. 0.5 mL of toluene and 2.5 mL of urea solution were added. Then stopper the flasks and was incubated for 2 h at 37 °C. After the incubation, with 50 ml of KCl solution was shaken for 30 min, then suspension filtrated. To a 25-mL test tube were added 1 mL of the filtrate with 9 Ml of deionized water, 5 mL of Na –salicylate/ NaOH solution and 2 mL of sodium dichloroisocyanide solution. 30 minutes later urease activity was determined colorimetrically at 690 nm and expressed as µg NH_4-N g $soil^{-1}$ $dwt2^{-h}$.

Arylsulphatase activity was detected according to Tabatabai and Bremner (1970a). 1 gram of moist soil was placed in to a 50-mL Erlenmeyer flask. 0.25 mL of toluene, 4 ml of acetate buffer and 1 ml of p-nitrophenyl sulphate solution were added. Then stopper the flasks and was incubated for 2 h at 37 °C. After the incubation and addition of 1ml of $CaCl_2$ (0.5M) and 4 ml of NaOH (0.5 M), suspension filtrated and Arylsulphatase activity was determined colorimetrically at 400 nm and expressed as µg ρNP g $soil^{-1}$ $dwt2^{-h}$.

Statistical analyses

Analyses of variance (ANOVA) were performed using the SPSS package. Significant differences within treatment combinations were assessed using Dancan's multiple comparison test.

Results and discussion

Soil physical and chemical properties taken in table 1.

Table 1. Soil physical and chemical properties.

Texture	Ec	pH	OC	$CaCO_3$	Fc	Available P	Available K	Available Fe	Available Zn	Available Cu	Available Mn	Available S
	dS/m			%				(mg kg^{-1})				
Loam	1.44	7.8	0.51	19.2	18.8	7	137	2.84	1.48	0.66	6.92	23.2
Silty clay	2.08	7.8	0.84	34.2	23.9	8.6	423	2.92	0.9	0.94	2.04	65.5
Clay	0.82	8	0.87	25.7	26.5	10.2	403	5.42	0.46	1.16	5.06	13.45

Sulfur effect on microbial biomass carbon and enzyme activities

Sulfur had significant effect on microbial biomass carbon, urease and arylsulphatase (Table 2). Sulfur effect on microbial biomass carbon was very clear. In three soils, microbial biomass carbon were decreased with increasing sulfur ($p<0.05$). The microbial biomass carbon in the control was generally higher than those in the other treatments. There was no significant difference between treatments (Fig. 1A).

Application of different levels of sulfur decreased arylsulphatase activity and significantly for S_3 (Fig.1B). The highest decrease in arylsulphatase activity was related to treatment S_3(668mgkg^{-1}) comparison with control ($p<0.01$). Urease activity

decreased with increasing sulfur at S_3 significantly ($P<0.01$). There was not much difference in the amount of urease activity between S_0, S_1 and S_2(Fig.1C).

Less study has been done on sulfur effect on biological activity. Application of sulfur reduced biological activity such as microbial biomass carbon, urease and arylsulphatase activity. Decreasing soil pH and increasing EC may be the main of reasons for decreasing of biological activities. Soil pH and EC was significantly influenced by sulfur (Soaud *et al.*, 2011; Orman and Ok, 2012). Soil biological activities are sensitive to pH change and pH was the most important factor influencing soil biological activities. Reducing soil pH has negative effect on soil micro flora and hence reduces microbial biomass carbon. Aciego Pietri and Brookes (2009) reported that biomass C was greatest above pH 7. There was statistically significant relationships between soil pH and biomass C ($R^2 = 0.80$, $p < 0.001$).

Table 2. Summary of analysis of variance of the effect of sulfur and compaction and their interactions on soil biological activities.

ANOVA source of Variation	df	Sum of square		
		MBC	Urease	Arylsulphatase
Sulfur	3	189309.69**	271.81*	5419.35**
Compaction	2	1964.86 n.s	309.78*	2187.22n.s
Sulfur * Compaction	6	85183.18**	116.02n.s	2466.54*

*, ** and n.s indicate the significance at the 0.05 and 0.01, probability levels and non-significance respectively; MBC, microbial biomass carbon, df, is degree of freedom.

Soil pH affected on enzyme activities by several mechanisms directly or indirectly. Soil pH can change the acidic or basic groups in the enzyme active center and the concentration of inhibitors, activators and the substrate in soil. Stability of soil enzymes to pH change is also highly dependent on soil properties (Frankenberger and Johanson, 1982). Changes in enzyme activities may reflect the changes in number and relative composition of soil microbes in relation to pH change.

In a study of phosphatase and arylsulphatase activities in wetland soils (Kang and Freeman, 1999), pH was found to be positively correlated with phosphatase activity. Acosta-Martı´-nez and Tabatabai (2000) also observed a significant and positive correlation between alkaline phosphatase and arylsulphatase activity with pH, the correlation coefficient were 0.89 and 0.66, respectively, and a negative correlation of acid phosphatase and pH with a correlation coefficient 0.69.

The sulfur oxidize by bacteria to sulfates(Jaworska, 1997) and produce acid enviroment and influence chemical and biochemical properties by enhancing or depressing availability of nutrients (Litynski and Jurkowska, 1982)for plants and microorganisms. Soil reaction, therefore, can affect the development of microorganisms not only directly, but also indirectly by modifying the availability of nutrients. This in turn has an indirect effect on enzymatic activity(Zellez *et al.*, 1990; Bardgett and Limans, 1995). Many enzymes are active in a slightly acid or inert environment. Sulfur produced a strongly acid environment, which was unfavorable to dehydrogenases, urease alkaline phosphatase and even acid phosphatase. Acosta-Martinez and Tabatabai (2000) reported that for the pH between 4.9 and 6.9, the activity of acid phosphatase in soil under agricultural crops was correlated negatively and the activity of other enzymes (glucosidase, galactosidase, amidase, urease, glutaminase, asparaginase and alkaline phosphatase) positively with the value of pH.

Soil salinity changes number and relative composition of soil microorganisms. Wang *et al* (2006) found that

reducing pH significantly lowered soil alkaline phosphatase activity, arylsulphatase activity and respiration. The relationship between soil biological activities and pH was well characterized by linear or quadratic regression models with R^2 values ranging from 0.57 to 0.99.

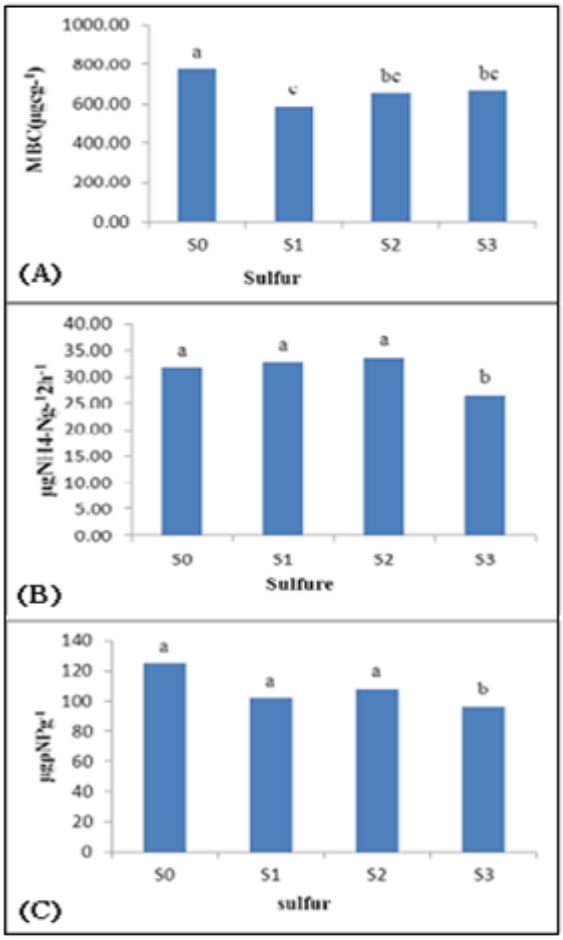

Fig. 1. Effect of sulfur on microbial carbon biomass (A), arylsulphatase activity (B) and urease activity (C).

Sardinha *et al* (2003) found that microorganisms activity limited in saline soil and thus microbial biomass carbon reduce. Decreasing microbial biomass carbon with increasing salinity were reported by other researchers (Tripathi *et al.*, 2006; Wichern *et al.*, 2006). Increasing soil salinity, combined with high soil pH, showed negative effect on all microbial indices including MBC, MBN, basal soil respiration, nitrification and net nitrogen mineralization. studies indicates that soils with the highest salinity level showed the lowest soil microbial biomass and activities, while soils with low salinity levels showed no effect on soil microbial indices.

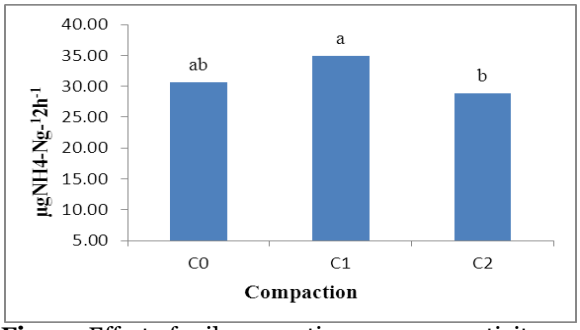

Fig. 2. Effect of soil compaction on urease activity.

Soil compaction effect on microbial biomass carbon and enzyme activities

Soil compaction had significant effect on urease activity ($p<0.01$) (Table 2) but compaction effect was not significant on microbial biomass carbon and arylsulphatase activity. Different levels of soil compaction had not significant on urease activity compared with control but there was significant difference in the amount of urease activity between C_1 and C_2. Urease activity was lower at C_2 than C_1(Fig. 2). Soil stresses such as compaction reduce soil aeration and infiltration, thus may adversely affect plant growth, through limiting root growth and microbial activity. Although soil compaction in low level has positive effect on plant growth and soil properties. Kaiser *et al* (1991) reported that soil compaction reduce microbial biomass carbon while other researchers found that soil compaction has not significant effect on microbial biomass carbon (Chen *et al.*, 2003; Jorden *et al.*, 2003; Li *et al.*, 2004).

Conclusion

Results from this study showed that soil compaction did not significantly influence MBC and, while sulfur significantly reduced MBC. Enzyme activities were affected by high levels of sulfur and soil compaction. Further investigations are required to examine the activity of enzymes and soil microbial biomass in response to higher level of soil compaction and sulfur.

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