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Microbial communities and their characteristics in a soil amended by nanozeolite and some plant residues: Short time *in-situ* incubation

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Abstract

Soil microbial communities and their related characteristics are an important agent for soil fertility, productivity, and sustainability. Also, they are useful indicators of soil quality and life index in agricultural systems. The objectives of this study were the effect of nanozeolite and plant residues on soil microbial communities and their characteristics and also, the assessment of incubation timing on soil microbial properties. Soil microorganisms are very important in the decomposition of plant residues. In this regard, the soil samples were treated by nanozeolite (0, 10 and 30% Weight), Alfalfa and wheat straw (0 and 5% Weight). The treated soil samples were incubated in lab condition for 90 days. The result of this study showed that Bacterial, Fungal, and Actinomycete populations increased by the addition of 30% of nanozeolite and 5% of plant residues, especially alfalfa straw. Also, the addition of nanozeolite and plant residues treatments improved MBC, BR, and SIR as microbial characteristics. These parameters increased after 30 days of starting incubation, then decreased until the 75th day and finally increased slightly on the 90th day. In fact, the addition of nanozeolite and plant residues into the soil had positive effects on improvement of carbon pools and increasing carbon sequestration in it. Applied nanozeolite and plant residues in soil, improved carbon pools and increased carbon sequestration in soil. Also the application of nanozeolite and plant residues especially alfalfa straw had positive effects on improvement of soil biological communities and characteristics.

Keywords: Actinomycete, Bacteria, Fungi, Biomass carbon, Respiration, Plant residue.

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Introduction

Plant residues, particularly cereal straw, which are mostly often returned to the soil are known as a potential source of bioenergy in arable farming in many countries (Su et al., 2006; Lal, 2007). One of the most important advantages of leaving higher densities of crop residues in the field is the increasing inputs of organic materials that benefit to the soil biological and biochemical properties (Guo et al., 2015). Recycling these residues can be led to the increase of soil organic matter (SOM) content, soil fertility, and also agricultural production. The decomposition, nutrient mineralization, microbial biomass (Baumann et al., 2013) and microbial community structure (Balser and Firestone, 2005) of soil can be related to the chemical properties of plant residues. In this regards, residues with lower carbon to nitrogen ratio (C:N) and lower concentrations of resistant compounds (e.g. lignin, condensed tannins, and insoluble waxes) lead to the faster decomposition (Chander and Joergensen, 2002; Grandy et al., 2013), increase carbon (C) mineralization (Fang et al., 2007) and increase microbial biomass (Jedidi et al., 2004; Nair and Ngouajio,

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2012) in soil. In contrast, plant residues with higher C:N ratio and higher concentrations of resistant compounds have more slowly putrefaction and nutrient cycling for instance C and even nitrogen (N) (Lal, 2004; Cordovil et al., 2007). Moreover, such residues could be caused an increase in relative abundance of soil microbial groups (e.g. fungi, bacteria, and actinomycete) that are adapted to nutrient-poor environments (Parham et al., 2003; Lucas et al., 2007).

Microorganisms play an integral unique role in organic matter decomposition, nutrient cycling and other chemical transformations in soil (Sinegani et al., 2009). Microorganisms also immobilize the significant amounts of C and other nutrients within their cells. The total mass of living microorganisms (microbial biomass) therefore has a central role as a source, sink, and regulator of the transformations of energy and nutrients in the soil. In agricultural systems, soil fauna can be important in organic matter decomposition, nutrient cycling, and SOM dynamics. In this regard, bacteria and fungi are mostly responsible for 90–95% of the total heterotrophic metabolism occurring in most soils (Fang et al., 2007; Sinegani et al., 2009). The microbial community and its diversity have been significantly positively correlated with a wide range of environmental variables such as soil pH, C:N ratio, and so on. Margesin et al. (2009) reported that fungi are able to grow and be active at low temperatures that this is a reason to increasing of fungal to bacterial ratio. Moreover, Djukic et al. (2010) provided evidence that the microbial community structure depends on the condition of decomposition in soil. The alterations in SOM pools due to modifications in the quantity and quality of available substrates are affected by changes in microbial community structures and activities (Balser and Firestone, 2005).

Zeolites (a Greek word meaning boiling stones) are generally found in rocks near active or extinct volcanoes, which means that zeolite deposits exist in many parts of the world (Montalvo et al., 2012). For many years, zeolites have attracted attention due to their physical and chemical properties (Colella and Gualtieri, 2007). According to the results of Gerrard et al. (2004) and Yeritsyan et al. (2008), zeolites are porous materials characterized by their ability to 1) lose and gain water reversibly, 2) adsorb molecules of appropriate cross-sectional diameter (adsorption property or acting as molecule sieves), and 3) exchange their constituent cations without a major change in their structure (ion-exchange property). Zeolites are known due to having many different potential applications in agriculture (Ramesh et al., 2010). Zeolites were commonly used as soil conditioners (Bansiwal et al., 2006). The study of Koci (1997) illustrated that zeolites were not usually toxic for organisms, who studying the possible toxic effects of water extracts of some cation zeolite forms on some water organisms. Chuprova et al. (2004) concluded that zeolite fertilizers have the beneficial effect not only on mobile humus substances of chernozem but also on the biological productivity of maize. Aminiyan et al. (2015a) reported that nanozeolite and alfalfa straw led to the increase of carbon pools and improvement of aggregation stability in the soils which treated and incubated in lab condition.

To date, many studies have demonstrated that the implementation of organic resources either from plants or animal sources improves SOM pools, supports rapid nutrient cycling through microbial biomass, as well as improves nutrient retention from applied mineral fertilizers in soils that located in both tropical and temperate regions. Organic resources are arguably short-lived especially in the tropics due to accelerated decomposition rates. Therefore, several applications of these organic materials are required in every cropping season. Currently, the interactive effect of nanozeolite and organic sources of nutrients like plant residues have been limitedly studied (Aminiyan et al., 2015a,b).

In this research, we investigated the synergistic effect of nanozeolite and some plant residues (alfalfa and wheat straws) as soil amendments on biological soil properties. The choice of these plant residues was based on their nutrient supply capabilities and wide usage as soil amendments on Iranian smallholder farms (Sinegani et al., 2009). We hypothesized that combining nanozeolite and some plant residues would significantly improve soil biological properties in comparison to lonely applied nanozeolite or even plant residues. In addition, nanozeolite interaction with alfalfa straw would be comparable to when combined with wheat straw in relation to the impact on biological soil parameters.

Material and Methods

Site description

This study was conducted in Hamedan province, the western part of Iran. This area is located between longitudes 47° 42' and 48° 45' E and latitudes 33° 28' and 34° 29' N. The climate of the region is semi-arid with a mean annual precipitation of 300 mm and a mean annual temperature of 10 °C. Agriculture is an industry and principal land use in Hamadan. The soil of studied area was classified as Typic Haplocalcids (Aminiyan et al., 2015a).

Nanozeolite and its characteristics

The nanozeolite was prepared from Fadak Institute, Isfahan scientific and technology park, Iran (Kamali et al., 2009). The morphology and the particle size of the nanozeolite examined by CM12 Phillips transmission electron microscope (TEM). X-ray diffraction (XRD) measurement was carried out using a D8ADVANCE diffractometer (BRUKER) with the Cuk_{α} (λ=1.54 Å) source at 40 kV and 40 mA. The agglomerated nanoparticles of nanozeolite were de-agglomerated by a milling process to obtain the product with 80–200 nm particle size.

Sampling, treatment and analysis of soil

The methods used to soil sampling, treatment and analysis were reported in (Aminiyan et al., 2015a). The treated and moistened soils were incubated in the lab condition (20-25 ^{o}C) for 90 days. After 1, 5, 10, 20, 30, 45, 60, 75 and 90 days of incubation, a portion of each soil was taken for the study of abundance of bacterial, fungi, actinomycete, and microbial characteristics (i.e. basal respiration (BR), substrate-induced respiration (SIR) and microbial biomass carbon (MBC))**.** Untreated soils were also incubated as controls.

Soil biological factors

To counting soil microorganisms, the plate count method was employed to estimate the number of bacteria, fungi, and actinomycete. The fresh soil suspension was serially diluted with saline buffer to obtain an appropriate number of colonies on each plate. The media of nutrient agar (NA), potato dextrose agar (PDA) and rose Bengal starch casein nitrate agar (RBSCNA) were, respectively, employed for culturing bacteria, fungi and actinomycetes (Alef and Nannipieri, 1995). Each dilution was plated in triplicate and the population was expressed as the number of colonies forming units (log CFU. $g^{-1}{}_{soil}$). After preparing each specific media in plates 0.1 ml of soil suspension of each serial dilution was spread across the plates (spread plate method). The incubation time at 28 ^{o}C for bacteria, fungi and actinomycete were 3, 4 and 14 days respectively (Alef and Nannipieri, 1995).

MBC was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Thirty grams (weighed dry equivalent) of moist soil was fumigated with ethanol-free chloroform for 24 hours. $CHCl_3$ was then removed and carbon was extracted from fumigated and non-fumigated soil samples with a 0.5 M K_2SO_4/soil ratio of 1:5 (v/w). After shaking for 30 min, suspensions were immediately centrifuged at 3000 rpm for 10 min. Then these soil suspensions were decanted and filtered. Thereafter, extractable carbon was determined in both of fumigated and non-fumigated soil. The microbial biomass carbon (MBC) was calculated according to the following equation (Vance et al., 1987):

$$MBC = \frac{EC}{KEC} \qquad \text{(Eq. 1)}$$

Where EC is the difference between extractable carbon from fumigated and non-fumigated samples and KEC = 0.38 (Dai et al., 2004).

Soil respiration (an estimation of the mineralization rate of the organic matter in the soil) was measured using the Isermeyer method (Isermeyer, 1952) at days 1, 5, 10, 20, 30, 45, 60, 75 and 90 after incubation. The 50 grams of soil samples (weighted dry equivalent) were moistened with distilled water to 80% of their water-holding capacities and put into closed jars containing 25 mL of 0.5 M NaOH. Incubations were carried out at 25 ^{o}C for 7 days for basal respiration (BR) and then titration of NaOH by 0.25 M HCl was done (Alef and Nannipieri, 1995). For substrate-induced respiration (SIR), a combination of 0.5 g glucose, 0.07 g NH_4Cl, and 0.01 g K_2HPO_4 was added to the soil, which was moistened and mixed carefully, and then incubated for 3 days with NaOH followed by titration with HCl. Titrations were calculated as (mg CO_2. $g^{-1}{}_{soil}$ day^{-1}).

Statistical data analysis

The experiment was a completely randomized factorial design with three replicates. Experimental data (i.e. the abundance of bacterial, fungi, and actinomycete, and also BR, SIR, and MBC) was subjected to analysis of variance and means compared by Duncan's new multiple range test (α = 0.01) by SAS Ver.9.2.

Results and Discussion

Table 1 illustrates that sand, clay, and silt contents were 69, 12 and 19% in studied soil respectively. Therefore, the soil texture was loamy sand. The soil was not saline (EC, 1.1 dSm^{-1}); equivalent calcium carbonate and pH values were 1.79% and 7.2 respectively, with low cation exchange capacity (CEC) 4.80 ($cmol_{+}$. $kg^{-1}{}_{Soil}$). And also, total organic carbon in pre-treatment soil was 3.41 $g.kg^{-1}$.

Table 1. Some of the chemical and physical properties of applied soil.

EC, dS. m^{-1}	pH	CEC, $Cmol^{+}. kg^{-1}_{Soil}$	Total organic C, g. kg^{-1}	CCE*	Sand, %	Clay, %	Silt, %
1.1	7.2	4.80	3.41	1.79	69	12	19

* Carbonate Calcium Equivalent

Table 2 presents some properties of applied plant residues. Alfalfa and wheat straw had a neutral pH (6 and 7.97), high OC (511 and 532 $g.kg^{-1}$) values and C:N (23.30 and 90.75) and C:P (85.20 and 123.50) ratios respectively. Some properties of applied nanozeolite present in Table 3. The pH value of nanozeolite was neutral, and nanozeolite is not saline. CEC in nanozeolite was 400.39 ($cmol_{+}. kg^{-1}_{soil}$). Figures 1 (A) and (B) demonstrate transmission electron microscopy (TEM) image of synthesized nanozeolite and X-ray diffraction (XRD) pattern of nanozeolite respectively.

Table 2. Some properties of applied plant residues in this study

	pH	EC, dS. m^{-1}	Total Organic C, g. kg^{-1}	Total N, g. Kg^{-1}	Total P, g. kg^{-1}	C/N	C/P
Alfalfa straw	6.00	9.50	511.00	22.00	5.98	23.30	85.20
Wheat Straw	7.97	4.30	532.00	7.00	4.31	90.75	123.50

Table 3. Some properties of applied Nanozeolite

EC, dS. m^{-1}	pH	CEC, $Cmol^{+}. kg^{-1}_{Soil}$
0.98	7.17	400.39

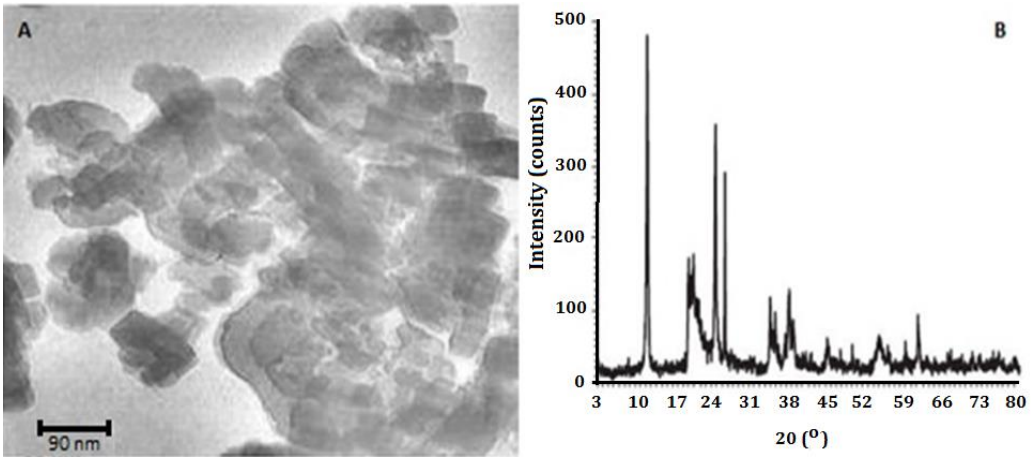

Figure 1. (A) TEM image and (B) XRD pattern of applied nanozeolite

According to the Table 4, it can be found that the addition of a greater percentage of nanozeolite, as well as wheat and alfalfa straws, particularly alfalfa straw, caused increasing microbial communities in all of the treatments. Bacterial colonies significantly increased ($p < 0.01$) with the addition of 30% nanozeolite and 5% alfalfa straw into the soil (N30A5 treatment). In other words, bacterial colonies increased (1.16 log $CFU.g^{-1}_{Soil}$) by the addition of 30% nanozeolite and 5% alfalfa straw than control. Also, a bacterial colony in N30A5 treatment was 0.76, 0.50 and 1.06 (log $CFU.g^{-1}_{soil}$) higher than N0W5, N10A5 and N30PR0 treatments respectively (Table 4).

Table 4. The abundance of Bacteria, Fungi, and Actinomycete (Log CFU. g^{-1}_{Soil}) colonies in all of the treatments

Treatment	**Bacteria, Log CFU. g^{-1}_{Soil}**	**Fungi, Log CFU. g^{-1}_{Soil}**	**Actinomycete, Log CFU. g^{-1}_{Soil}**
Control	8.79±0.130 *g	5.08±0.274 g	7.02±0.182 g
N0A5	9.19±0.138 e	5.84±0.281 e	7.58±0.206 e
N0W5	8.99±0.146 f	5.56±0.287 f	7.36±0.209 f
N10PR0	8.90±0.145 g	5.16±0.279 g	7.12±0.210 g
N10A5	9.45±0.136 c	6.48±0.275 c	8.04±0.205 c
N10W5	9.32±0.137 d	6.27±0.276 d	7.83±0.208 d
N30PR0	8.89±0.133 g	5.20±0.275 g	7.16±0.206 g
N30A5	9.95±0.127 a	6.91±0.273 a	8.46±0.207 a
N30W5	9.64±0.128 b	6.67±0.274 b	8.28±0.206 b

*. Mean ± Standard deviation. N0A5 (0% nanozeolite+5% alfalfa straw), N0W5 (0% nanozeolite+5% wheat straw), N10PR0 (10% nanozeolite+0% Plant Residue), N10A5 (10% nanozeolite+5% alfalfa straw), N10W5 (10% nanozeolite+5% wheat straw), N30PR0 (30% nanozeolite+0% Plant Residue), N30A5 (30% nanozeolite+5% alfalfa straw), N30W5 (30% nanozeolite+5% wheat straw).

Figure 2 reveals the change trend of the abundance of bacterial colonies during on the 90 days of incubation period. Accordingly, it shows that bacterial colonies increased with the passage of time from the 1st day of the incubation period (9.19 log CFU. g^{-1}_{soil}) to the 30th day (9.31 log CFU. g^{-1}_{soil}). Then it decreased until on the 75th day (8.91 log CFU. g^{-1}_{soil}) and finally it increased again until on the 90th day (8.96 log CFU. g^{-1}_{soil}). In this regard, (Yi et al., 2013) reported that the abundance of total bacterial colony was increased in soil following slurry incubation from the 1st day of the experiment (8.8×10^8 CFU. g^{-1}_{soil}) until the 5th day (9.5×10^8 CFU.g^{-1}_{soil}) and then it decreased until the 20th day (9×10^8 CFU. g^{-1}_{Soil}) and finally it increased until the 30th day (9.2×10^8 CFU. g^{-1}_{soil}) of the experiment period.

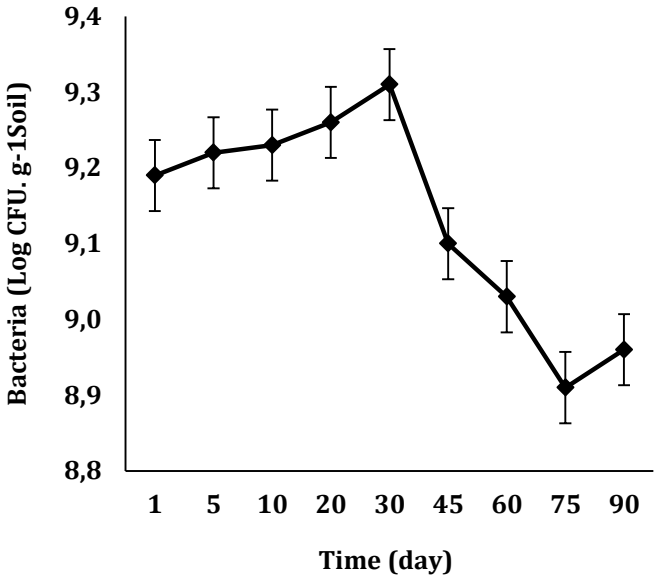

Figure 2. Abundance of bacteria (log CFU. g^{-1}_{soil}) with the passage of time

Abubaker et al. (2013) reported that the soil incubation with residue-A, residue-B, and cattle slurry for 120 days resulted in significant shifts in bacterial community structures compared with the control within each soil, especially in the sandy soil. Furthermore, they concluded that bacterial community structure in the cattle slurry treatment was significantly different from that in both of residue treatments in all soils.

Table 4 shows that the addition of nanozeolite, wheat, and alfalfa straws into the soil interestingly increased fungal colonies abundance, especially in (N30A5) treatments. In other words, the fungal community had the highest abundance in this treatment in comparison with the other treatments ($p < 0.01$). Hence, fungal colonies (1.83 log CFU. g^{-1}_{soil}) had more interestingly increased in (N30A5) treatment than control. And also as shown in Table 4, this treatment was 1.07, 0.43 and 1.71 (log CFU. g^{-1}_{soil}) higher than N0W5, N10A5, and N30PR0 treatments respectively. Figure 3 shows that fungal colonies abundance increased correspondingly during the first 30 days of the incubation period. In other words, fungal colonies increased from (5.73 log CFU. g^{-1}_{soil}) on the1st day up to (6.37 log CFU. g^{-1}_{Soil}) on the 30th day. Then fungal colonies decreased until the 75th day (5.59 log CFU.g^{-1}_{soil}) and finally they were increased until the 90th day (5.7 log CFU. g^{-1}_{soil}).

In another study, (Abubaker et al., 2013) found similar cultural bacterial and fungal species composition in low input and conventional agriculture. Marschner et al. (2003) reported that the addition of organic and inorganic amendments can be led to the significant changing in the biological and chemical properties of soil. Accordingly, they concluded that long-term addition of organic amendments at a low rate may increase bacterial biomass while having no effect on fungal biomass. They also reported the fundamental importance of OC for soil microorganisms. Different amendments affected the bacterial and eukaryotic community structure through their effect on OC and the C:N ratio of the soil.

Changes in microbial community composition are often observed after the addition of organic or inorganic amendments but organic matter with a high C:N ratio is only slowly degraded by microorganisms (Garcia-Pausas and Paterson, 2011). In a number of short-term studies, it has been shown that organic amendments increased microbial community and microbial biomass (Wang et al., 2013; Ninh et al., 2015). An increase in soil microbial abundance and enzyme activity was also observed after the addition of either NPK fertilizer or farmyard manure in a long-term study (Su et al., 2006; Lucas et al., 2007). However, Crecchio et al. (2001) reported no changes in bacterial community structure after the addition of municipal solid waste compost in a short-term study. A higher frequency of bacteria and their activity in soil against fungi is because the fungi are more important for macroaggregate formation because of their hyphae as compared with bacteria. So that, fungi predominantly proliferate in larger pores among macro- and micro-aggregates; whereas bacteria reside in smaller pores within microaggregates (Ventorino et al., 2012). In other words, they concluded that the larger size of pores may make fungi more vulnerable to predation, whilst small pores provide refuge for bacteria against predators.

Table 4 indicates that actinomycete colonies abundance increased with the addition of nanozeolite and also wheat and alfalfa straws into the soil, especially in Nanozeolite and alfalfa straw treatments. In other words,

(N30A5) treatment caused a significant increase in the abundance of actinomycete colonies (*p <0.01*). So this treatment had more increased actinomycete colonies (1.44 log CFU. g^{-1}_{Soil}) in comparison with the control and also 0.88, .42 and 1.30 (log CFU. g^{-1}_{Soil}) higher than N0W5, N10A5 and N30PR0 treatments respectively (Table 4). As shown in Figure 4, the abundance of actinomycete colonies increased during the first 30 days of the incubation period. In other words, their colonies increased from (7.95 log CFU. g^{-1}_{Soil}) to (8.3 log CFU. g^{-1}_{Soil}) on the 1st day and the 30th day respectively. From the 30th day to on the 75th, they decreased (7.74 log CFU. g^{-1}_{Soil}) and finally they increased once again on the 90th day (7.76 log CFU. g^{-1}_{Soil}). The results of this study reveal that the abundance microbial colonies in the N30A5 treatment were significantly different from other treatments. A small, but significant, separation was also observed between N30A5 and N30W5.

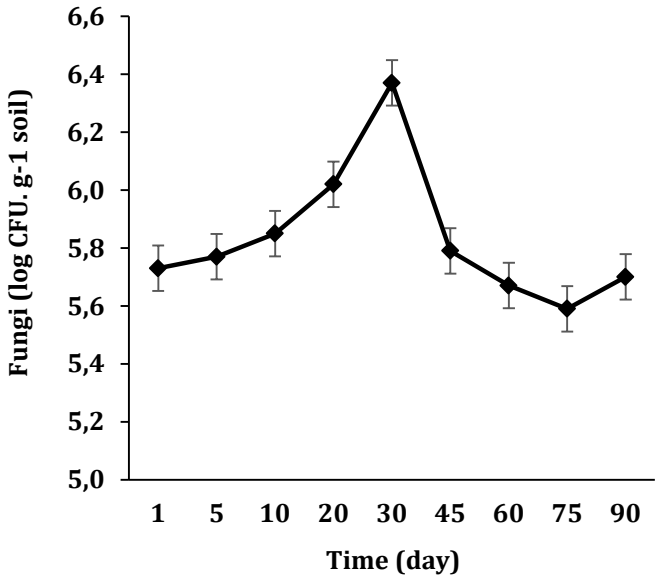

Figure 3. Abundance of Fungi (log CFU. g^{-1}_{Soil}) with the passage of time

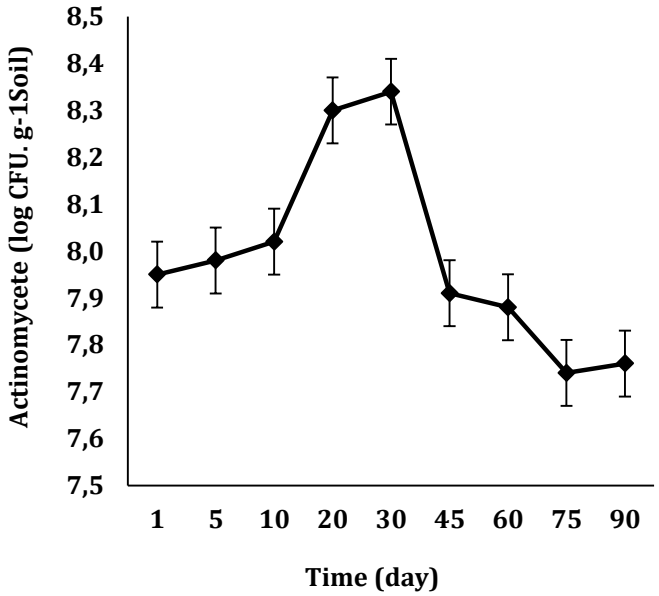

Figure 4. Abundance of Actinomycete (log CFU. g^{-1}_{Soil}) with the passage of time.

Andronikashvili et al. (2012) reported that under the influence of clinoptilolite-containing tuffs in red and podzolic soils, which are weakly populated by actinomyces, their quantity sharply increases. This contributes to the sterilization of soil from undesirable microflora since it is known that these microorganisms are antibiotics protecting soil against bacterial microflora (Andronikashvili et al., 2012). Mühlbachová and Šimon (2003) concluded that the microbial populations could respond to zeolite amendment in different ways. Zeolites have high ability to bind with humic acid by the action of the surface extra-framework cations. This ability markedly enhanced, if the zeolitic material enriched by divalent cations especially Ca^{2+} (Capasso et al., 2005). Therefore, the zeolite can be led to the improvement of soil structure through formation of micro- and macroaggregates (Aminiyan et al., 2015a). Likewise, Andronikashvili et al. (2012) concluded that in the presence of 5% zeolites in the soil, both qualitative and quantitative change of microflora is observed because mycolytic bacteria were dominated which led to lysis and/or devouring of mold fungi. Also, they found that the increasing of zeolite quantity (10-15%) to the soil, can be caused much more diverse in the microbial community. This is easily explained by the continuous inputs of organic material in the treatments with residue, which serve as C source for energy synthesis.

Soil management practices, such as the cover cropping and compost application can be enhanced the biological activity of soil such as bacterial, fungal and actinomycete diversity and populations (Nair and Ngouajio, 2012). Other studies have also shown high soil microbial diversity and populations following manure or compost application (Tu et al., 2006; Treonis et al., 2010). Manure 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; Liu et al., 2010).

According to Table 5, whenever the application of higher percentage of nanozeolite and also plant residues especially alfalfa straw, the MBC, BR and SIR parameters increased in the whole treatments. MBC value was maximum in (N30A5) treatment; so that its MBC value (0.381 gr C. kg^{-1}_{soil}) was higher than control and it had a significant difference (*p <0.01*) with other treatments (Table 5). Figure 5 demonstrates that MBC increased

from the 1st day (0.491 gr C. kg^{-1}_{soil}) until the 30th day (0.545 gr C. kg^{-1}_{soil}) of the incubation period then it decreased until the 75th day (0.453 gr C. kg^{-1}_{soil}) and finally, it increased until on the 90th day (0.461 gr C. kg^{-1}_{soil}). It could be due to the pre-incubation period that may have contributed to microbial growth, initiated by the favorable environmental conditions (25°C) and strengthened by soil watering and organic matter provision. During the early phase of the incubation, the soil microbial biomass preferentially incorporated labile C pools derived from the added residue over the native and more recalcitrant material. Jorge-Mardomingo et al. (2013) concluded that the trend of MBC changes was similar to the results of the present study. Previous studies have shown that MBC was increased during the initial days of the incubation period but it decreased in the later days of the experiment and then MBC increased at the end of the experiment with the addition of plant residue (Jedidi et al., 2004; Raiesi, 2004; Fereidooni et al., 2013).

Table 5. MBC, BR and SIR values in all of the treatments

Treatment	MBC, gr C. kg^{-1}_{Soil}	BR, mg CO_2. g^{-1}_{Soil}. Day^{-1}	SIR, mg CO_2. g^{-1}_{Soil}. Day^{-1}
Control	0.289±0.034 * **e**	0.22±0.017 e	0.74±0.20 g
N0A5	0.421±0.035 f	0.27±0.018 e	0.84±0.21 g
N0W5	0.389±0.033 g	0.24±0.016 e	0.80±0.19 g
N10PR0	0.482±0.035 e	0.65±0.020 d	1.85±0.21 f
N10A5	0.640±0.036 b	0.82±0.023 c	2.21±0.23 d
N10W5	0.590±0.040 c	0.78±0.021 cd	2.12±0.24 e
N30PR0	0.523±0.039 d	1.01±0.022 b	2.61±0.19 c
N30A5	0.670±0.038 a	1.18±0.019 a	2.96±0.20 a
N30W5	0.632±0.040 b	1.13±0.018 ab	2.86±0.22 b

*. Mean ± Standard deviation. The same letter is not significantly different at $p < 0.01$ using Duncan's LSD.
N0A5 (0% nanozeolite+5% alfalfa straw), N0W5 (0% nanozeolite+5% wheat straw), N10PR0 (10% nanozeolite+0% Plant Residue), N10A5 (10% nanozeolite+5% alfalfa straw), N10W5 (10% nanozeolite+5% wheat straw), N30PR0 (30% nanozeolite+0% Plant Residue), N30A5 (30% nanozeolite+5% alfalfa straw), N30W5 (30% nanozeolite+5% wheat straw).

Generally, the addition of residue as a new C source enhanced soil microbial activity, causing to a significantly greater accumulation of MBC at the beginning of the incubation. A lack of residue addition, in each treatment with lower contents of residue, decreased the MBC significantly compared to higher residue amended treatments. This is because recalcitrant C pools in the treatments which have lower contents of residue and also were not able to supply the same quantity of energy to the microbial community. For instance, microbial activity is stimulated and decomposition occurs more readily when residues with a lower lignin content and a low C:N ratio, such as alfalfa, are added to the soil. In fact, it was expected that residue type changes would strongly influence MBC because microbial biomass is a sensitive short-term indicator that can detect these changes.

Chander and Joergensen (2002) also found that soil microbial biomass increased after the addition of zeolite amendment. Microbial biomass and community are primarily affected by soil structure and substrate availability. Microbial processes take place at the scale of soil aggregate, which is essentially a porous structure that varies both spatially and temporally. This is because soil organic matter located within soil aggregates which are physically protected from biodegradation, aggregates enhance carbon sequestration and soil structural stability (Aminiyan et al., 2015a). Also, they found that zeolitic amendment firstly promotes aggregates formation and its associated C incorporation caused by direct and indirect binding agents. In general, microbial biomass increases with aggregate size (from micro- to macroaggregates) because of increasing OM amount. In contrast microaggregates, the macroaggregates occlude more manure-derived SOC due to the physical entrapment of particulate OM (Bichel et al., 2016).

BR and SIR values increased with the addition of a greater percentage of nanozeolite and plant residues, hence, these indicator values were maximum in (N30A5) treatment, as BR and SIR values were higher (0.96 mg CO_2. g^{-1}_{Soil} Day^{-1}) and (2.22 mg CO_2. g^{-1}_{Soil} Day^{-1}) in this treatment than the control respectively. In another word, this treatment had a significant difference ($p < 0.01$) with other treatment (Table 5). In the studied soil treatments, the differences of SIR contents between the whole of treatments may be related to differences in carbon quality of the added materials but were of a small magnitude. These results are in agreement with a previous pot experiment conducted by Chen et al. (2012), which showed that the application of cattle manure had significantly positive effects on soil biological properties. So that it let to increase soil carbon's pool, and microbial biomass, as well as caused changes in microbial community structure. As shown in Figure 6, the trend of changes in BR and SIR with over time was similar to that of MBC; BR increased from

(0.638 mg CO_2. g^{-1}_{Soil} Day^{-1}) on the 1st day to (1.08 mg CO_2. g^{-1}_{Soil} Day^{-1}) on the 30th day. Then it began to decrease up to the 75th day (0.438 mg CO_2. g^{-1}_{Soil} Day^{-1}) and at the end of the incubation period (90th day) it increased (0.458 mg CO_2. g^{-1}_{Soil} Day^{-1}) once again. The trend of SIR was upwards from the 1st day (0.74 mg CO_2. g^{-1}_{Soil} Day^{-1}) until the 30th day (2.27 mg CO_2. g^{-1}_{Soil} Day^{-1}) of the experiment. Then its value began to decrease until the 75th day (1.59 mg CO_2. g^{-1}_{Soil} Day^{-1}) and finally its value (1.62 mg CO_2. g^{-1}_{Soil} Day^{-1}) increased on the 90th day of the incubation period (Figure 6).

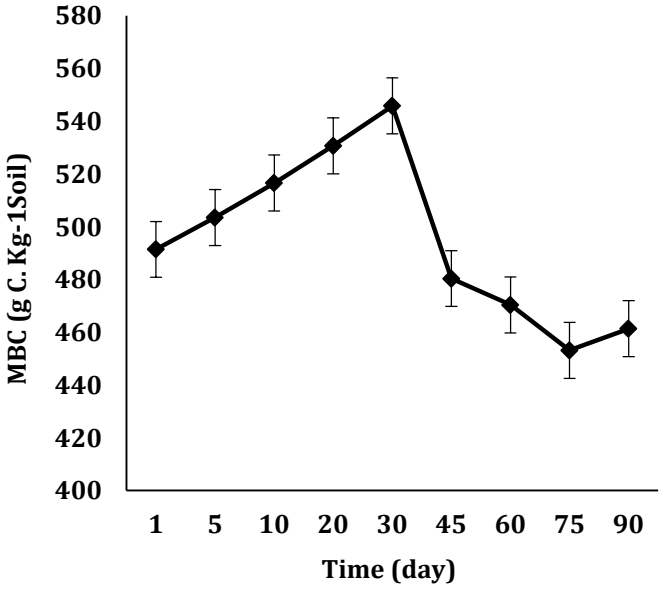

Figure 5. Microbial biomass carbon (MBC) (gr C. Kg^{-1}_{soil}) changes with the passage of time

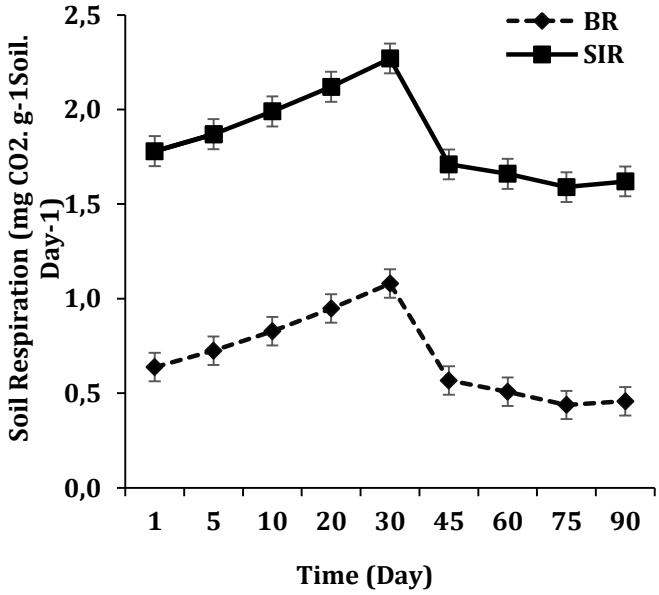

Figure 6. Basal respiration (BR) and Substrate-induced respiration (SIR) (mg CO_2. g^{-1}_{soil}. Day^{-1}) changes with the passage of time.

Jorge-Mardomingo et al. (2013) reported that BR had a similar trend to the results of the present study during the incubation period. The cause of enhancement of frequency and function of microorganisms in the first 30 days of incubation is increasing readily biodegradable materials in soil. And also, decreasing of them after 75 days of incubation is due to the reduction of the availability of simple biodegradable ingredients in it. And also, again the enhanced frequency and function of microorganisms in the final days of the incubation period is probably due to the adaptation of soil microorganisms to deficiency of simple biodegradable ingredients in soil (Chen et al., 2012; Grandy et al., 2013). Organic farming systems with compost applications had 34% higher microbial biomass than treatments which did not get any manure application (Fließbach et al., 2007). Also, they reported higher basal respiration in organically managed production systems compared to unfertilized control plots.

Abubaker et al. (2013) also suggested that both biogas residues and cattle slurry reduced bacterial substrate-induced respiration (SIR) in organic soil than in sandy and clay soil. However, Chen et al. (2012) demonstrated in a pot experiment that the addition of biogas residues can be stimulated and increased both basal respiration (BR) and substrate-induced respiration (SIR), during shorter incubations. The assessment of fifty years of crop residue management showed that residue management had a limited impact on heterotrophic respiration, metabolic diversity of soil bacteria and soil cold-water extracted carbon (Buysse et al., 2013). In fact, soil heterotrophic respiration increased in the initial days of the incubation period but in the continuation of the experiment it decreased and finally it increased at the end of the experiment, these results suggested that both short and long-term processes are likely to occur concurrently in response to residue management (Fereidooni et al., 2013). Govaerts et al. (2007) observed significant differences 15 years after the initiation of a long-term tillage and residue management to improve soil properties such as low tillage with the addition of residue into the soil. These results were in line with those of the present study.

Conclusion

Soil microbial communities and their related characteristics are an important agent for soil fertility, productivity, and sustainability. Also, they are useful indicators of soil quality and life index in agricultural systems. Soil microorganisms are very important in the decomposition of plant residues. The achieved

results from this study showed that bacterial, fungal and Actinomycete populations increased by the addition of nanozeolite and plant residues especially alfalfa straw as the (N30A5) treatment were more effective than the other treatments. Also, the (N30A5) treatment was more effective in increasing and improving MBC, BR, and SIR than the other treatments. According to the trend of MBC, BR, and SIR changes, these characteristics increased in the initial days of the experiment until the 30th day but they declined in the continuation of the experiment period until on the 75th day, and then, the increased slightly on the 90th day once again. In fact, the application of nanozeolite and plant residues into the soil improved carbon pools and increased carbon sequestration in it. Also the application of nanozeolite and plant residues especially alfalfa straw had positive effects on improvement of soil biological communities and characteristics.

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