Journal of Animal and Veterinary Advances 11 (17): 3140-3147, 2012
ISSN: 1680-5593

Change of Soil Protozoa Community Structure under Different Farming Practices

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Abstract: A study was conducted in 13 years of Organic Fertilizer (OF) and nofertilizer (U) field experiment in a winter wheat-summer maize rotation agroecosystem in the North China plain. The study was also included an Greenhouse (GH) experiment in cucumbers and kidney bean rotation in the North China Plain. Researchers aimed to evaluate the changes in soil physico-chemical parameters and the abundance and community structure of soil protozoan, under Greenhouse (GH), Organic Fertilizer (OF) and nofertilizer (U) different treatments, compared to difference management measure soil as the control (U). Soil organic matter content, available potassium (K) and hydrolyzable N were significantly higher in the plots under the GH treatment than under the other treatments. Available Phosphorus (P) content of OF was significantly higher than control treatment. After continuous many years organic application treatment and GH treatment which both increased the abundance soil protozoa. Both types of organic fertilizer and GH reduced the relative abundance of amoeba whereas increased relative abundance of flagellate in experiment. However, relative abundance of amoeba in the three treatments have occupied the overall majority. This result was positively related to the high level of phosphorus in this experiments which probably suppressed fungi thus increasing the food resources for bacterivorous protozoa, especially in flagellate. The GH and organic fertilizer treatments remarkably increased the overall abundance of protozoa. While the GH and organic fertilizer application treatments did not increase the relative abundance difference groups of soil protozoa, respectively especially amoeba increasing the relative abundance of flagellate, the increase in flagellate reflected enhanced biological activity and functioning of the bacterial decomposition pathway especially in the food web of this treatment.

Key words: Protozoan community structure, soil food web, organic fertilizer, nofertilizer, flagellate

INTRODUCTION

In the soil ecosystems, reservoirs of minerals and nutrients, detoxifies pollutants and modifies soil structure were provided by soil food web (Van Straalen and Gestel, 1993; Doran and Parkin, 1994). The interaction of the components of the soil food web which will drive organic matter decomposition, plant productivity, quality of crops and nutrient cycling and so on function. Understanding the function of soil food webs is an important priority for soil ecologists. Whereas, change of species can alter the goods and services provided of soil food web (Tilman *et al*., 2006), so biodiversity plays important roles in soil food webs because it maintains not only agricultural productivity but also ecosystem health, soil biodiversity was also hold that resistance to stress and disturbance (Brussaard *et al*., 2007; DuPont *et al*., 2010).

Protozoan occupy multi-trophic levels of the soil food web also play important roles in soil functioning, interact with many other organisms by fragmenting decaying organic matter, dispersing microbial propagules and consuming and being consumed by other components of the soil fauna (Couteaux and Darbyshire, 1998; Sanchez-Moreno and Ferris, 2007). Soil protozoan also

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show varying activity in different microhabitats plays important roles in ecosystems, the carbon and nitrogen cycles of soils by regulating decomposition rate and specific metabolic pathways (Couteaux and Darbyshire, 1998). There is convincing evidence respire about 10% of the total carbon input, mineralize 20-40% of the net nitrogen (Foissner, 1994) which was also represent an important component of soil food web and there is evidence that soil protozoan maintains both agricultural productivity and ecosystem health (Brussaard *et al*., 2007; DuPont *et al*., 2010). Food-web interactions among soil biota have significant effects on the quality of crops by mediating the availability of water, nutrients and certain.

Soil biota diversity is the basis for their multiple functions and roles. Among the soil protozoan, flagellate as effective predators can play important roles in agroecosystems which may regulate their prey populations are essential elements of the microbial loop in soils (Schwarz and Frenzel, 2003). Ciliate and amoeba as also actively predators of soil but land use changes often lead to a modification of protozoan populations and numbers of species (Foissner, 1994) which cannot easily escape stress conditions (Schwarz and Frenzel, 2003). These three major groups of soil protozoa are therefore indicators of soil conditions, ecological disturbance and anthropogenic impact (Kuikman *et al*., 1990; Salamon *et al*., 2006).

Agricultural intensification can result in important changes in soil biological communities which may decrease biodiversity, loss of key species and change in trophic relationships (Beare *et al*., 1997; Giller *et al*., 1997; Wood *et al*., 2000). These changes were also evidenced, they promoting the maintenance of soil fertility by adding fertilizers rather than through nutrient recycling, finally decrease internal function of soil food web (Altieri, 1999). Whereas the mechanism is still little understood.

The application of organic fertilizer to soils is an integral tool for sustainable agriculture as evidenced by the increasing interest in optimizing this strategy (Lue *et al*., 2000; Treonis *et al*., 2010). As researchers all know, organic fertilizer application will increase the abundance of various components of the soil food web such as the soil microbial community, protozoa and microbivorous nematodes (Saison *et al*., 2006; Salamon *et al*., 2006; Carrera *et al*., 2007; Forge *et al*., 2008). As far as sustainable agriculture is concerned, application of organic fertilizer is generally regarded as a more sound cultivation than chemical fertilizer application (Maeder *et al*., 2002; Bengtsson *et al*., 2005). Organic fertilizer was generally regarded as a nutrient source and will increase the abundance and activity of soil biota For instance, soil microorganisms (Peacock *et al*., 2001), protozoans (Altieri, 1999), nematodes (Hu and Cao, 2008), collembolans (Altieri, 1999) and earthworms (Doran and Werner, 1990) and so on. The specific objective of this research was to investigate impact of long-term different fertilization application on soil protozoa. In order to obtain the effects of long-term fertilization on soil protozoa abundance compared to a non fertilized system that served as a control assess differences impact organic and chemical fertilizer amendments soil on protozoa community composition and identify the relationship between components of the soil protozoa community and soil abiotic conditions under different fertilization treatments.

MATERIALS AND METHODS

Site description: The study is located at Qu-Zhou Experimental Station (36°52'N and 115°01'E), China Agricultural University. The station is in a continental temperate monsoon zone and the climate in the region is warm, sub-humid and consists of summer rainfall and dry cold winters. The mean annual temperature is 13.2°C and ranges from a minimum of -2.9°C in January to a maximum of 26.8°C in July, mean annual precipitation is 542.7 mm, of which 60% occurs from July to September and the annual non-frost period is 201 days. The mean annual evaporation is 1841 mm and is more three times annual rainfall, so spring drought is very severe. The soil at study site is an improved silt fluvo-aquic soil where the soil amendment engineering started in 1970's (De-Hui, 1990).

Experiment design: Experiment was conducted in farmland and vegetable greenhouse. The farmland experiment was originally set up in 1993 with different fertilizer regimes under cultivation system of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) (Cao *et al*., 2011). In 1997, another set of experiment with the same treatments was launched again to repeat the 1993 fertilizer trial as a parallel experiment. The greenhouse experiment was set up in 2002 year with treatments of organic fertilizer, chemical fertilizer and combination of both, under cultivation of tomato, cucumber and bean (Yang *et al*., 2009). There are three replications for each treatment in farmland and greenhouse.

In this study, three treatments were selected from greenhouse experiment and farmland experiment which was launched in 1997 as follows: greenhouse with organic compost fertilizer (GH). The organic compost including 100.5 kg N ha^{-1} consisting of 60% (w/w) straw (wheat straw in June, maize straw in October), 30% chicken dung, 5% cotton seed-pressed trash and 5% bran was applied at 15 ton ha^{-1} to the OF plots and incorporated into the soil by cultivation Organic compost Farmland (OF) (The organic compost of OF treatment is same to GH treatment) and unfertilized farmland as control (U). While the protozoa community was investigated in 2010, the greenhouse experiment was conducted for 8 years and the farmland experiment was conducted for 13 years.

Soil sampling: Soil samples were collected from three replication plots of the each treatment in June and October 2010 using a 3×20 cm auger in 0-20 cm depth. At every plot, 8 cores of soil were taken and mixed into a soil sample then put it into a plastic bag immediately. A total of 18 samples were collected at each sampling date. The samples were taken to the laboratory and kept in refrigeratory at 4°C waiting for soil physical and chemical property analysis and protozoa measurements.

Soil and protozoa measurement: Soil moisture in each sample was determined by weight loss after heating at 105°C for 24 h and expressed as a percent dry weight. Sub-samples from each replicate were analyzed for Soil Organic Matter (SOM), available N, available Phosphorus (P), available Potassium (K) and pH. SOM was determined using the potassium dichromate external heating method (Blakemore *et al*., 1981). Hydrolyzable N was determined by the Alkaline-Hydrolyzable Diffusion Method. Available P was extracted with 0.5 mol L^{-1} $NaHCO_3$ (soil: solution ratio = 1:20) and measured with the Olsen method (Blakemore *et al*., 1981). Available K was extracted with 1 mol L^{-1} NH_4Ac (soil: solution ratio = 1:10) and measured with the flame photometry method. Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ slurry (soil: solution ratio = 1:2.5) using a glass electrode. Protozoa abundance was measured by Most Probable Number (MPNP Method which was used to estimate the population abundance of flagellate, amoeba and ciliate with three level ten fold dilution cultivation technology.

Data analysis: The effect of different treatments on soil physico-chemical characteristics and protozoa community were analysed with ANOVA, to test the difference in parameter values between soils at each site, Least Significant Difference (LSD) were used to evaluate differences between separated means. Differences obtained at levels of $p<0.05$ were considered significant. The treatments which are indicated in the tables by the same letter. All statistical analyses were performed by SPSS (11.5) Software package. A Canonical Correspondence Analysis (CCA) was conducted using CANOCO for Windows 4.5 (Microcomputer Power, Ithaca, USA), it was in order to elucidate the relationships between protozoan populations, different fertilization regimes and soil physico-chemical parameters. In the CCA, the abundance data for three major groups and environmental variables were biplot scaled, there were used Monte Carlo test to test the null hypothesis of no significant effect of different treatments or physico-chemical parameters on protozoan populations.

RESULTS

Soil physico-chemical characteristics: The soil physico-chemical parameters were shown in Table 1. In GH plots, organic matter content, hydrolyzable N, available P and available K were significantly higher than in OF and U plots. And in OF plots, these four parameters were significantly higher than in U plots. Meanwhile, pH values in GH, OF and U plots were at the same level, no obvious difference existed. It implied that as soil organic carbon and nitrogen increased from unfertilized farmland to organic fertilized farmland and greenhouse, the pH values did not decrease significantly, signal of acidification had not yet appeared as it usually happened in other intensive agricultural area although, the available P content in GH plots were 6 fold as in OF plots and 86 fold as in U plots.

Protozoa community abundance: The abundance of soil protozoa community was significantly affected by farming practices and land use types as shown in Table 2 ($p<0.05$).

Table 1: Soil physico-chemical parameters in treatments

Treatments	SOM (g kg^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	pH
GH	50.99±3.24^{a}	280.49±11.81^{a}	559.63±17.28^{a}	572.90±26.65^{a}	7.73±0.26^{a}
OF	23.07±1.66^{b}	157.75±6.660^{b}	93.28±10.55^{b}	213.4±31.930^{b}	7.70±0.17^{a}
U	15.54±0.30^{c}	122.86±6.360^{c}	6.54±0.370^{c}	79.54±7.090^{c}	7.96±0.05^{a}

GH = Greenhouse; OF = Organic fertilized farmland; U = Unfertilized farmland; SOM = Soil Organic Matter; N = Hydrolyzable nitrogen; P = Available Phosphorus; K = Available potassium. Different letters in a column denote significant differences ($p<0.05$)

Table 2: Mean protozoa abundance and functional groups abundance (10^3 ind g^{-1} dry soil) and their frenquancy (percentage of total number) in treatments. Soil samples were collected in June and October 2010 in depth of 0-20 cm

		Flagellate		Ciliate		Amoeba	
Treatments	Total abundance	Abundance	Frenquancy (%)	Abundance	Frenquancy (%)	Abundance	Frenquancy (%)
GH	206.70±42.60^{a}	35.08±10.343^{a}	16.97	12.20±3.62^{a}	5.90	159.42±41.15^{a}	77.12
OF	149.61±60.48^{b}	11.02±2.0400^{b}	7.37	6.40±5.06^{b}	4.28	132.18±57.11^{a}	88.35
U	33.61±14.94^{c}	1.99±0.4700^{c}	5.91	0.86±0.44^{c}	2.56	30.77±14.63^{b}	91.54

GH = Greenhouse; OF = Organic Fertilized Farmland; U = Unfertilized farmland. Different letters in a column denote significant differences ($p<0.05$)

In Greenhouse (GH), the total number of protozoa per game dry soil was the highest, followed by OF treatment and the lowest was in U treatment which was only 16% of the abundance in greenhouse. Soil protozoa community consisted of three functional groups: flagellate, ciliate and amoeba. The abundance of these three groups were significantly different in treatments ($p<0.05$) but showed the same rank as the total number of protozoa. This rank was consistent with the organic matter content and nitrogen levels in treatments, more carbon and nitrogen, higher protozoa abundance.

Protozoa community structure: Three functional groups' frequency (percentage of the total number) indicated the protozoa community structure. As shown in Table 2, amoeba was the dominant population in this protozoa community and its frequency was between 77.12~91.54% among treatments. Flagellate and ciliate were the minority, their frequency were 5.91~16.97 and 2.56~5.90%, respectively.

From U to OF and GH treatment, frequency of flagellate and ciliate increased while the frequency of amoeba decreased with the richness of nitrogen. The phenomena was strange and was not consistent with common sense. According to the trophic resources of amoeba which was the predator of bacteria while nitrogen increased in soil, population abundance of bacteria would increased, so that the population abundance of amoeba should increased as well. Why the predicated phenomena did not appear in this investigation? Researchers carefully analyzed the nitrogen fractions in soil samples and found that phosphorous was the first factor shifted remarkably among treatments. Regressive analysis was made between soil phosphorous level and frequency of protozoa functional group as shown in Fig. 1. While soil available phosphorous concentration increased from 10-600 ppm level, the percentage of flagellate and ciliate increased but amoeba percentage decreased.

Relationships between treatments, protozoa functional group and soil physico-chemical properties: The correlation relationship between soil physico-chemical parameters and protozoa functional groups was analyzed by SPSS 13.0 Software. Results indicated that the frequency of flagellates was positive correlated to available N, P and K with the coefficient as 0.472, 0.568* and 0.585*, respectively. Morever, the frequency of amoeba was negative correlated to these parameters. For pH value, the scenario was different which the frequency of amoeba was positive related to while flagellate and ciliate were negative correlated to. Results of the in the ordination diagram with a high level explanation (Fig. 2). The eigenvalue in first axis reached as high as 81.2% and in second axis was 2.3%. In this diagram, amoeba was at the center of the plot as generalist with no preference for any treatment. Three treatments were well separated from each other and location as determined by all the biotic and abiotic parameters, two farmland treatments, U and OF were located in second and third quadrant, respectively. One was in the upper quadrant near to pH value and another was in the lower quadrant. However, the treatment in Greenhouse (GH) was in opposite of these two farmland treatments, it was close to the contents of soil organic matter and available N, P and K. This result was consistent with the effect of treatments on soil physico-chemical properties (Table 1).

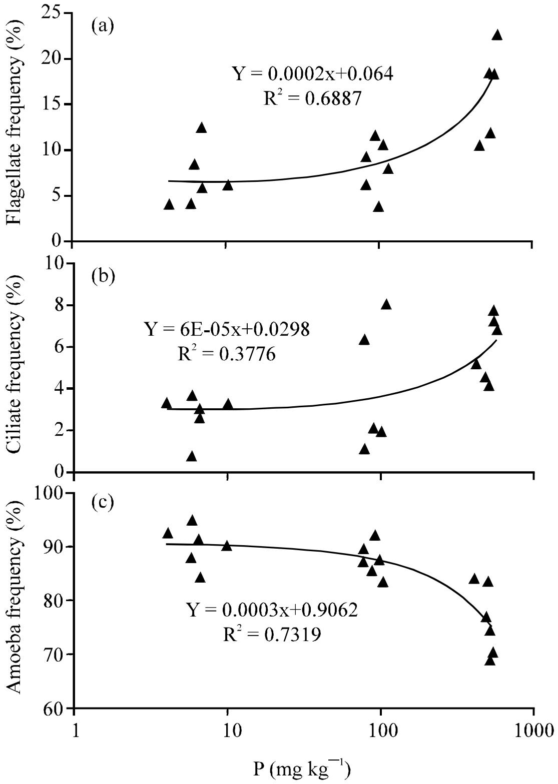

Fig. 1: Regressive relationship between protozoa functional groups' frequency and available phosphorus concentration in soil were shown. a) the regressive relationship between flagellate frequency and P concentration; b) the regressive relationship between ciliate frequency and P concentration; c) the regressive relationship between amoeba abundance and P concentration and amoeba abundance

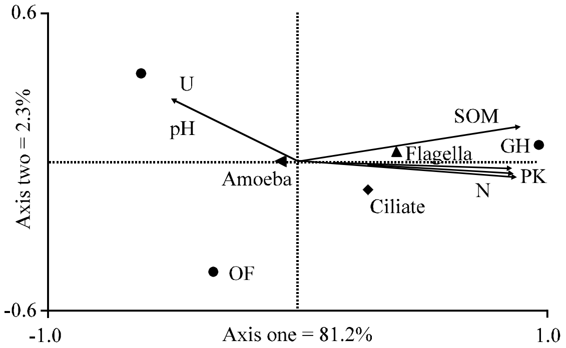

Fig. 2: The relationships between physico-chemical parameters, treatments and the abundance of three functional groups. The soil physico-chemical parameters are represented by arrows and the treatments by black circle. GH = Greenhouse. OF = Organic Fertilized farmland. U = Unfertilized Farmland. SOM = Soil Organic Matter; N = Hydrolyzable Nitrogen; P = Available Phosphorus; K = Available Potassium

DISCUSSION

Flagellate abundance under the different fertilizer treatments: In the present study, flagellate was much more abundant of protozoa group in soil which is consistent with the findings of Finlay *et al.* (2000), Finlay and Fenchel (2001) that flagellate accounted for 63.47 and 60.01% of the total soil protozoa. However, those researchers reported that abundance of soil protozoan are dominated by amoebae with lower abundance of flagellates and sparse populations of ciliates under arid region soils (Rodriguez-Zaragoza and Garcia, 1997; Robinson *et al.*, 2002; Zaragoza *et al.*, 2007). Considering the different soil environment, researchers thus speculate that the dominance of amoeba in the agroecosystem of experiment treatments may be attributed to the abundance of the different temperate area with a different climate and a clear distinction between the four seasons or tolerant species as well as disturbance-tolerant species.

Both organic and chemical fertilizers increased the total abundance of soil protozoan (Treonis *et al.*, 2010) which was mainly attributed to the apparent increase in flagellate at experiment treatments which consistent with the findings of Ning and Shen (1998) that compost had a effect on protozoan abundance and protozoa community structure. Effect of different fertilizers application on the soil protozoa showed that total abundance of protozoa, three major community abundance and amoeba relative abundance increased significantly with increasing year by year whereas flagellate relative abundance was decreased, flagellate relative abundance was little change (Bai, 2006). Surprising to find that available P concentrations were unsimilar in the organic and conventional treatments. Available P levels in the GH plots were higher than in the OF and U plots, respectively. Because of supply of phosphorus was greater than the amount required for crop growth and may be resulting in P accumulation in the tillage soil layer. It is possible that P levels may be among the factors affecting flagellate abundance and relative abundance, probably indirectly through their influence on the soil microflora.

There is convincing evidence that while exchange for carbon from plant hosts, Arbuscular mycorrhizal fungi can facilitate plant uptake and transport of less mobile soil nutrients (such as phosphorus) (Thingstrup *et al.*, 2000; Jakobsen *et al.*, 2001; Chen *et al.*, 2005). Whereas with higher P levels in soils, the development of AM fungi is controlled by soil P availability, the significance of P transport by mycorrhizal fungi declines (Baath and Spokes, 1989; Hamel *et al.*, 2008). It can thus be inferred that under GH, OF and U regimes, fungi could have decreased in relative abundance, bacteria increased in relative abundance, resulting in increased food resources for flagellate. In the present study found that the abundance of flagellate that feed on bacteria such as major heterotrophism flagellate, increased under a high P level.

Ciliate abundance in the different fertilizer treatment: Ciliate have been found to have sparse populations of ciliates (Finlay *et al.*, 2000; Finlay and Fenchel, 2001) although, the abundance of ciliate was also increasing with increase soil fertility, relative abundance was very low but more heterogeneous feeding habits. Fertilization treatments had positive impacts on the abundance of ciliate: organic fertilizer significantly increased the abundance of ciliate but not significantly increased relative abundance of ciliate under different treatments. The reasons for sparse populations of ciliates, they maybe come down to two major ones: for one thing soil ciliate is more lower groups in soil protozoa and for another feeding habits was also relatively complicated as following, carnivorous, bacterivores, fungivorous and omnivorous (Ning and Shen, 1998). However, at present there is insufficient available information to support this conclusion.

Amoeba abundance: A trend of increasing abundance of Amoeba with increase P was observed in difference treatments which may be attributed to more wheat residues in the soil. Amoeba have similar feeding habits to flagellate as naked amoeba and testate amoebae, naked amoeba mainly preys on bacteria whereas testate amoebae preys on bacteria and algae, accounting for almost all (Ning and Shen, 1998). However, the trend of decreasing relative abundance of amoeba with increase P was observed in the study but amoeba dominating abundance of soil protozoan and may be attributed to different soil environment and time of planted which consistent with findings of Zwart *et al.* (1994). Organic fertilizer may benefit the populations of Amoeba due to the high nutrient supply. Because the flagellate are small volume and sort of life-cycle which were more favorable for upgrowth than amoeba under the high nutrient supply, so led to limiting relative abundance of amoeba.

CONCLUSION

The long term use of different fertilizer treatments affects edaphic biocenoses by altering the organic inputs and soil microhabitat. In summary, two conclusions were reached from the results. The first is that soil fertilization increased the total abundance of protozoa and abundance of three major groups, possibly due to increase food resources for them arising from the application of fertilizers. The second conclusion is that the organic fertilizer increased the relative abundance of flagellate, possibly as a result of facilitation heterotrophic flagellate surface soil in the presence of phosphorus-rich resources meanwhile suppression of mycorrhizal fungi and increase relative abundance of bacterial. This may be a limitation of the application of both organic and chemical fertilizers. Overall, it can be simply concluded that soil fertilizer application benefited the abundance and relative abundance of soil flagellate whereas for the relative abundance of amoeba the opposite was the case. The increased soil protozoan abundance supports evidence from parallel studies that organic amendment strongly influenced the wider biological community and enhanced decomposition and nutrient cycling in the soil food web by strengthening both bottom-up and top-down processes.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (30970536), the Key Discipline Foundation of Ecology in Beijing Municipality (Grant No. XK10019440) and China Agricultural University Scientific Fund (2009 TD15). Researcher thank Professor Zhi-Ping Cao for her valuable sugggestion on an early draft of this manuscript andthank Yun-feng Chen and Gue Zhang.

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