EFFECT OF ORGANIC AND INORGANIC FERTILIZERS ON SOIL MICROBIAL BIOMASS AND MINERAL N DURING CANOLA (*BRASSICA NAPUS* L.) DEVELOPMENT

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1 INTRODUCTION

Although small in mass, microbial biomass is among the most labile pools of organic matter and thus serves as an important reservoir of plant nutrients such as N and P. Microbial biomass is a sensitive indicator of change resulting from agronomic practices and other perturbations of the soil ecosystem. Its size and activity is directly related to the amount and quality of carbon and other nutrients available from plant residues, organic amendments and root exudates (Wang and Klassen, 2007). Other factors influencing microbial communities are soil moisture and temperature and physical disturbance of the soil.

Application of dairy-feedlot manure as a supplementary source, have been shown to maintain or increase soil carbon and microbial biomass improving soil quality than that achieved with chemical fertilization (Mandal et al., 2006). However, information on the effects of dairy-feedlot manure, as an alternative source, on soil biological properties during active crop growth stages and its relationship with seed yield is sparse. Hence, the present study was undertaken to assess the impact of short-term application of combined mineral fertilizer and farm yard manure compared to single applications of each on soil biological traits during two critical stages of canola growth and their relationships with crop yield in a temperate agro-ecosystem.

2 MATERIALS AND METHODS

A two year experiment was conducted in 2004-2005 and 2005-2006 at the Research Station of Shahid Beheshti University in Savadkooh (36º 40′ N and 53° 10′ E, 1200m above sea level), a mountainous area in the southern zone of Mazandaran Province in the north of Iran.

The experiment was conducted as a randomized complete block design with four replications. Treatments included 0 (F_0), 50 (F_{50}), 100 (F_{100}), 150 (F_{150}) kg N ha^{-1}, 100 kg N ha^{-1} as urea + 50 kg N ha^{-1} as manure ($F_{100}M_{50}$), 50 kg N ha^{-1} as urea + 100 kg N ha^{-1} as manure ($F_{50}M_{100}$), and 150 kg N ha^{-1} as manure (M_{150}). Treatments were located on the same plots during both years. Half of the urea was applied at planting and the remaining was manually side-dressed at the beginning of stem elongation. Beef cattle feedlot manure (collected during October 2004 and 2005) was applied in both years. It was incorporated into the 15 cm topsoil by disking 2 weeks prior to planting. Manure application was based on the assumption that 35 and 20% of total N in manure would become available during the first and second years after its application, respectively. Based on this assumption, oilseed rape received 43 (31.3 t ha^{-1} in the first year and 11.7 t ha^{-1} in the second year), 28.6 (20.9 t ha^{-1} in the first year and 7.7 t ha^{-1} in the second year) and 14.6 t ha^{-1} (10.4 t ha^{-1} in the first year and 4.2 t ha^{-1} in the second year) of non-composted manure in treatments M_{150}, $F_{50}M_{100}$ and $F_{100}M_{50}$, respectively. Manure application was on dry weight basis. Information on OC, total and inorganic N, P, EC, pH and manure moisture is presented in Table 1.

Plots were 2.1m wide (7 rows with 0.30m row spacing) and 5m long. Oilseed rape cv. Hyola was overseeded on 6 and 16 November 2004 and 2005, respectively, and thinned to 66 plants m-2 at the three-leaf stage. Oilseed rape was cultivated under rainfed conditions, so that no irrigation was applied. Plots were kept weed-free during the growing season by hand weeding. No fungicide or insecticide was applied since there was no serious problem due to diseases or insects.

Soil sampling was conducted at two physiological stages of canola, stem elongation and flowering, on April and May 2006, respectively. At each sampling, five soil cores (2.5cm diameter, 15 cm depth) were taken from each plot, and mixed as a composite sample. The composite samples were placed in plastic bags and transported to the laboratory, where field moist soil was sieved (2mm mesh size), homogenized and stored at 4 °C.

Total organic carbon in soil was determined by dichromate oxidation. The microbial biomass C and N was estimated using the chloroform fumigation extraction method. The soils were extracted with 2M KCl and inorganic-N (NH4 + NO3) in the extracts was measured by steam distillation.

All data were analyzed statistically using the GLM procedure of SAS. The Duncan multiple range test (DMRT) set at 0.05 was used to determine the significance of the difference between treatment means.

3 RESULTS AND DISCUSSION

3.1 Natural condition in experimental area

The total annual rainfall of the region was 738 and 712 mm in 2004-2005 and 2005-2006, respectively, of which 540 mm and 578 mm occurred during the crop growing period (October-June) of 2004-2005 and 2005-2006, respectively. Mean seasonal air temperature during fall, winter, spring and summer of 2004-2005 was 14, 8.4, 17.9 and 22.3ºC, respectively. These values were 16.2, 8.1, 19.8 and 25.2ºC for 2005-2006. The soil is classified as Alfisol. The soil texture of the experimental plots was clay loam. Soil available P (18 mg kg^{-1}) and exchangeable K (479 mg kg^{-1}) both appeared to be adequate for production of 3 t ha^{-1} of oilseed rape grain yield. Information on some other soil characteristics including organic carbon (OC), total N, P, electrical conductivity (EC) and pH is presented in Table 1. The experimental site had been under paddy rice cultivation for several years up to 1999 and had been left fallow since then.

TABLE 1 **Chemical analysis of soil (0-15 cm layer) and manure applied in 2004-2005 and 2005-2006.**

Source	OC (g kg^{-1})	Inorganic N (mg kg^{-1})	Total N (g kg^{-1})	P (ppm)	EC (ds m^{-1})	pH	Moisture (%)
Soil	13.3	20	2.43	18[a]	1.07	7.8	
Manure (2004)	220	1028	13.7	3500[b]	7	6.4	40
Manure (2005)	200	1293	15.6	4200[b]	6	7.0	45

[a] , [b] Available and total phosphorus, respectively.

3.2 Soil microbial Biomass

The microbial biomass carbon (MBC) was low in the control and increased significantly in plots amended with manure and fertilizer at both growth stages (Table 2 and 3). Application of the sub-optimum level of N (35% N) significantly decreased MBC and the microbial biomass nitrogen (MBN). The soil microbial biomass, which consist 1–5% of total soil organic carbon, can be used as an effective early warning of the improvement or deterioration of soil quality as a result of different management practices (Wang and Klassen , 2007). In the present study, the values ranged between 2 and 2.4%, with the lowest and highest values being associated with the control and manure amended plots, respectively. Supplying 100% of the canola N requirement with manure, resulted in higher microbial biomass than supplying 35 and 65% of crop N requirement with manure. This result is inconsistent with the observations of Hopkins and Shiel (1996) on grassland, who reported that the microbial biomass was greater in soils following annual additions of both farmyard manure and inorganic NPK than in soil following additions of inorganic fertilizers or farmyard manure alone. These researchers suggested that the readily metabolizable C and N in organic manure, not only increasing root biomass and root exudates as a result of greater crop growth but also greatly contributed to biomass increase. The disagreement may be related to the fact that in Hopkins's and Shiel's experiment manure was applied as a supplementary source while in the present study manure was applied as an alternative source of fertilization.

The observations on the effect of physiological stages indicated that MBC and MBN were subjected to the time of sampling. The stem elongation stage of canola had a higher positive impact on soil MBC and MBN than the flowering stage. Microbial biomass is a small but very dynamic component of soil organic matter fluctuating with the weather, crop, input and season (Mandal et al., 2006). It might be suggested that the interaction effect of treatments and rhizosphere conditions induced by plant growth stages played an important role in the enhancement of microbial biomass to a more around stem elongation. On average, shoots transport about half of the C fixed in photosynthesis to the root (Nguyen, 2003). Thus, higher plant growth due to short-term application of manure might would stimulate more below ground flux of C fixed by photosynthesis. The release of organic C from roots into the soil might be regarded as a pool of reduced C that no longer contributed to dry matter production. However, it is well established that rhizodeposits stimulate biological activity as found in the present study as well, and benefits plants by enhancing nutrient availability.

TABLE 2 **Soil organic carbon (OC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC/OC ratio, MBC/MBN ratio and nitrogen mineralization as affected by different fertilizer treatments at stem elongation stage of canola**

Treatments	Mineral N mg kg^{-1}	MBC/MBN	MBN mg kg^{-1}	MBC mg kg^{-1}
F_0	14.3e	4.38b	64d	280e
F_{50}	24.7d	4.04b	73c	295d
F_{100}	36.6c	4.1b	81b	331c
F_{150}	66a	4.3b	75b	322c
$M_{50}F_{100}$	62.3ab	5.7a	77b	441b
$M_{100}F_{50}$	54b	5.5a	79b	437b
M_{150}	33.2c	5.5a	87a	480a

TABLE 3 **Soil organic carbon (OC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC/OC ratio, MBC/MBN ratio and nitrogen mineralization as affected by different fertilizer treatments at flowering stage of canola**

Treatments	Mineral N mg kg^{-1}	MBC/MBN	MBN mg kg^{-1}	MBC mg kg^{-1}
F_0	17.7c	3.5c	60d	252e
F_{50}	16.7c	4.2bc	72c	301d
F_{100}	18c	4.4b	73b	323c
F_{150}	18.3cb	4.6b	69b	320c
$M_{50}F_{100}$	20.2b	5.6a	70b	395b
$M_{100}F_{50}$	21.3b	5.4a	75b	405b
M_{150}	24.5a	5.4a	80a	431a

3.2 MICROBIAL BIOMASS C/N RATIO

MBC to MBN ratio in soil showed the significant effect of fertilizer treatments (Tables 2 and 3). The significant decrease in this ratio in chemical fertilizer treatments may be due to changes in microbial community structure and size as a result of changes in the inputs of metabolized N (Gomez et al., 2006). Higher MBC to MBN indicates the chance of more N immobilization by microbes than its availability by mineralization or its limitation in manure.

3.3 N MINERALIZATION

The highest mineral N (66 mg kg-1) was observed due to the interactive effect of 100% N treatment and stem elongation (Tables 2 and 3). Similar to MBC and MBN, mineral N in soil was higher at stem elongation than flowering stage. Supplying 35, 65 and 100% of the canola N requirement with manure-N compared to supplying the same level of fertilizations with 100% urea-N decreased mineral N at stem elongation stage by 6, 33 and 50%, respectively. Briar et al. (2007) found that the conventional farming system had more N in the mineral pools whereas the organic farming system had higher N in the microbial biomass pool, indicating shifts in nitrogen deposition between the two systems. Inversely, the above mentioned treatments in the present study increased mineral N by 10, 16 and 34%, respectively, at flowering stage. Presumably, there was a higher release of plant-available N from the soil microbial biomass toward the end of the experiment, e.g via higher temperature, more dry condition or predators (Ferris et al., 1996) as indicated by the fact that there was enough N during the late growth stages to sustain adequate canola yields in the $F_{50}M_{100}$ and $F_{100}M_{50}$ treatments, in spite of lower total N uptake compared to F_{150} treatment (Sabahi et al., 2009). The significant positive relationship that exists between mineral N and microbial biomass at the flowering stage supported this hypothesis (R^2= 0.78).

A positive and significant relationship (p=0.01) was found between soil mineral N and seed yield at stem elongation (R^2= 0.89). Stepwise regression was used to find out the critical biological and biochemical factors associating with higher grain yield of canola. It was observed that mineral N at stem elongation (R^2 = 0.89), and MBN (R^2 = 0.085) at flowering were the two most important traits affecting canola seed yield. Wang and Klassen, 2007 also reported a high correlation between plant biomass and grain yield in tomato with the level of soil microbial N. Canola growth rate and demand for N during the stem elongation stage is high.

4 CONCLUSION

Mineral N and microbial biomass in soil were improved by the integrated applications of chemical fertilizer and manure at the flowering stage. This resulted in enough N during the later growth stages to sustain adequate canola yields in the integrated treatments, in spite of lower N uptake in these treatments compared to the chemical treatment. Mineral N at stem elongation and microbial biomass N at flowering stage were highly associated with seed yield. Application of cattle manure over a 2-year period resulted in significant increase in C/N ratio in microbial biomass which suggests that the changes in soil microbial community and functional importance of such changes should be investigated under manure-based and chemical fertilization systems.

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REFERENCES

Briar S S, Grewal P S, Somasekhar N, Stinner D, Miller S A 2007. Soil nematode community, organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from conventional to organic management. Applied Soil Ecology 37, 256-266.

Ferris H, Venette R C, Lau S S 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems and their impact on soil fertility. Applied Soil Ecology 3, 161–175.

Gomez E, Ferreras L, Toresani S 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technology 97, 1484–1489.

Hopkins D W, Shiel R S 1996. Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. Biology and Fertility of Soil 22, 66-70.

Mandal A, Patra A K, Singh D, Swarup A, Masto R E 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology 98, 3585-3592.

Nguyen C 2003. Rhizodeposition of organic C by plants: mechanism and controls. Agronomie 23, 375–396.

Sabahi H, Soufizadeh S, Mirzaei Talarposhti R 2009. Zn deficiency in winter oilseed rape (*Brassica napus* L.) is counteracted by N fertilization. Acta agriculturae, Section B, Soil and Plant Science. 59, 317-325.

Wang Q R, Li Y L, Klassen W 2007. Changes of soil microbial biomass carbon and nitrogen with cover crops and irrigation in a tomato field. Journal of Plant Nutrition 30, 623-639.