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ORIGINAL ARTICLE

Green Manure, Mycorrhizas and Soil Fertility

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ABSTRACT

Because some semi-arid soils under monoculture have lower biological activity, an increased use of the biological potential of these soils to counter the challenges of food production problems is proposed. To determination role of green manure rotation and fallow on semi-arid soils fertility, a field experiment was carried out at the research farm of the Seed and Plant Improvement Institute, Karaj (Iran) involving monocultures and intercrops of Persian and berseem clover with and without arbuscular mycorrhizal (AM) fungi *Glomus mosseae* inoculation and a land fallow soil treatment as control.. Clovers as well as AM fungi compared to fallow improved soil fertility, total N, soluble phosphorus, total C, Soil microbial C biomass and soil nitrogen-fixing rhizobia. Result showed inoculation clover rotation with mycorrhizal is a better option to improve soil fertility.

Key words: arbuscular mycorrhizal, soils fertility, green manure rotation

Introduction

In Iran as some parts of world, soil quality of farms declined by intensive agriculture. This study tried to measure potential soil biological indicator (mycorrhiza) in different intercropping systems to improve soil quality. Forage legumes can be important components of sustainable crop rotations. Legume benefits to subsequent crops are attributed to the addition of N and to non-N rotation factors such as disease and weed control, and to improved soil water holding capacity (Shrestha *et al.,* 1988). Berseem clover (*Trifolium alexandrinum* L.) and Persian clover *(Trifolium resupinatum* L.) where are annual leguminous forage or cover crop species well adapted to semi-arid conditions of the Mediterranean areas. They are high-yielding, nutritious, cool-season forage crops (Knight, 1985) grown in pure stands or in mixtures with annual grass species for over winter grazing and for harvested forage in spring (Martiniello, 1999; Stringi *et al.,* 1987). they are important winter annual forage species for in a crop rotations in the Mediterranean region and Iran but Iranian farmers don't encourage choosing this clover species as main crop, therefore investigated potential of this clover species as summer annual forage source when spring seeded is important .

Low P availability limits plant growth in many alkaline soils. P deficiency is mainly caused by strong adsorption of $H2PO_4^-$ to calcium (Ca), which turns large proportions of total P into forms that are unavailable to plants. The improvement of P nutrition of plants has been the most recognized beneficial effect of mycorrhizas.

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The last mechanism could lead to access to inorganic and AM plants have been reported to improve nutrition of the other macronutrient N. AM fungi may be important for the uptake of ammonium (NH_4^+), which is less mobile than nitrate (NO_3^-) and where diffusion may limit its uptake rate. Although nitrate is much more mobile than ammonium (uptake is regulated through mass flow), AM fungi may be important in nitrate uptake in Mediterranean and (semi-)desert ecosystems. The effect of AM fungus on soil microbiological properties recorded in the literature is inconsistent (Hodge, 2000; Johansson *et al.,* 2004).

The hypothesis of this research were (1) legume crop rotation (Persian and berseem clover) will improve soil fertility (2), intercrop system will further affect soil fertility differently,(3) AM fungi will further affected soil fertility (4), legume crop as well as AM fungi can use as mean to improve soil.

Materials and methods

Study area

Field experiments were conducted at the research farm of the Seed and Plant Improvement Institute, Karaj (54 º50_ N, 55 º35_ W and 1312 m above sea level) during dry seasons (2007). The experimental sites are located in a semi-arid region with mean annual rainfall of 270mm. The rainfall is confined to five-and-a-half months from November to February with practically negligible rainfall during spring and no rainfall in summer (May-August). The average maximum temperature from May to July is very high (26.5-44 8C) with a mean of 27 8C. In October, temperature falls to a minimum of 12 8C. It reaches a maximum of 48 5C in August. The soils of site are classified as medium black, clayey, shallow (15-20 cm depth). The soil is characterized by low organic carbon (1.2). The treatments were include 5 cropping systems, stand ratios of 1:0 (84:0 plants m^{-2}) 3:1(63:21 plants m^{-2}), 1:1(42:42 plants m^{-2}), and 1:3(21:63 plants m^{-2}) 0:1(0:84 plants m^{-2}) of berseem clover to Persian clover (BP) that inoculated with *Glumus mosseae* and a land fallow soil treatment as control. The inoculated (750 spores cm^{-3} of inoculum) dosage was 360 g of inoculums m^{-2}. Since mycorrhizal spore propagules extracted from the native soil were extremely low (1-2 per kg), no attempt was made to fumigate the soil. Each stand ratio treatment had 84 plants. Field establishment rates of Persian and berseem clover seeds were 40 and 70% of seed sowing rate, respectively. Clover seeding rates were based on this research, but ultimately seedlings were thinned to 84 plants m^{-2} in the appropriate ratio. Plots were fertilized 30 kg N, 15 kg P2O5, 15 kg K2O and 5 kg ZnSO4 per hectare. Sowing date was 18 may. The necessary plant protection, irrigation and other management practices were followed during crop growth. Pesticides weren't applied. Clovers were grown with irrigation. Irrigations were given at 10-day interval; in each irrigation 65 mm water was applied through flooding. No serious incidence of insects or diseases was observed. Crops were harvested twice, once before date of corn and twice before wheat sowing in this area by sickle from ground level and the first, second and total above-ground biomass yield was recorded. Samples were cut from an inner plant area of 2 m^2 at 5 to 7.5 cm above soil level and the total above-ground biomass was recorded. After the fall forage yield, root and crown was returned to the harvest area within each plot and evenly distributed. Crop fall forage, were incorporated into the soil using a disc.

The mycorrhiza colonization rate of both clovers species was assayed before the cutting. The mycorrhiza colonization assessment was carried out using the method described by Brundrett *et al.* (1996). Root were stained in trypanblue, and mycorrhiza colonization levels determined using the gridline intersect method of Giovanetti and Moss (1980).

Soil sampling

Colony form unit (CFU) numbers of various groups of rhizosphere N_2-fixing bacterial species were estimated by plate counts of aliquots from serial 10-fold dilutions 2 weeks before clovers harvesting on 10 July. The root systems of 10 plants were gently separated from the bulk soil and the soil adhering to the roots was considered rhizosphere soil. Rhizosphere soil was shaken in Ashby's liquid medium minus carbon source, and suitable dilutions were plated on Jensen's agar to give a count of N2-fixing bacterial. Three replicate plates were inoculated from each dilution and one dilution series was prepared per soil suspension. The plates were incubated at room temperature in the dark and the colonies emerging were counted. The morphology of the colonies on the plates was checked and the numbers of CFU were counted.

The method of Jenkinson and Powlson (1976) was used to Soil microbial C (MBC) after regrowth harvesting. Briefly, each soil was weighed into duplicate beakers and fumigated with ethanol-free chloroform at 25_C for 24 hours. After removal of chloroform vapour by repeated evacuation, the soils were inoculated with 1% unfumigated soils and placed in 1.5-L Mason jars. This was undertaken following the method of Vance et al. (1987). The soils were then extracted with 0.5 M K_2SO_4. Controls were prepared by extracting soil without fumigation. The soil suspension was filtered through a Whatman no. 42 filter paper (Whatman Ltd., UK). Total organic C content in the soil extracts was measured with a dichromate digestion method.

Microbial biomass C was calculated from the difference in extractable organic C between the fumigated and unfumigated soil, as follows:

MBC _ 2.64 *F*EC [1]

Where FEC refers to the difference in extractable organic C be tween fumigated and unfumigated treatments; and 2.64 is the proportionality factor of MBC released by fumigation extrac- radiation (Vance et al., 1987).

Soil organic matter by the Walkley-Black wet oxidation method (Walkley and Black, 1934), total nitrogen by the Kjeldahl method (Bremner and Mulvaney, 1982).

Statistical analysis

Statistical analyses by ANOVA were performed using SAS software (1990). The significance of difference between treatments was estimated using the LSD range test with a 0.05 if a main effect or interaction was significant.

Results and discussions

Clover plants were colonized in all treatments that were inoculated with AM fungi *G. mosseae* (43%). Non-inoculated treatments (control) registered only 5-6% colonization. There was no significant effect ($P \le 0.05$) of clover ratios on mycorrhiza colonization rate (Table 2).

Table 1: Soil characteristics measured before crop establishment.

Experiment sites	Percentageof total C (0-25 cmSoil depth)	Percentageof total N (0-25 cmSoil depth)	available P(0.5 mol/ LNaHCO3)mg/kg	Soil microbial C biomass	N2 fixers (g-1soil*$\times 10^3$cells)
Soil characteristics measured before crop establishment.	1.2	0.027	12.21	267.9	18.7

Table 2: Mycorrhiza colonization rate%, Forage yield of single or mixed cropping clovers (P and/or B). Means±s.e., p<0.001.

Treatments		Mycorhiza Colonization	Forage dry matter
		%	Kg ha^{-1}
	1:0(B)	22.8	6227.60d
	3:1(B:P)	24.8	6375.60b
Cropping system	1:1(BP)	24.8	6617.60a
	1:3(BP)	25.1	6289.60c
	0:1(P)	24	5552.6
		43.5a	6224.70a
		5.12b	6200.50b
Arbescular mycorrhiza× Cropping system		NS	NS

The yield of clovers was expressed as dry weight for various treatments at each (2) cutting as well as the sum of total dry weight. Clover ratio and AM fungi had significant ($P \le 0.05$) effect on total forage yield (Table 2). Total forage yield was greatest with a 1:1 ratio of berseem to Persian clover and was further increased by AM inoculation (Table 2).

Clover rotation compared to fallow and before clovers establishment enhanced total soil organic C, total N, Soil microbial biomass C, soil nitrogen-fixing rhizobia and was further increased by AM inoculation (Table 3).

Table 3: Soil characteristics measured after crop harvesting. Means±s.e., p<0.001.

Treatments	Percentageof total C (0-25 cmSoil depth)	Percentageof total N (0-25 cmSoil depth)	available P(0.5 mol/ LNaHCO3)mg/kg	Soil microbial C biomass	N2 fixers (g-1soil*$\times 10^3$cells)
fallow (control)	1.17c	0.026d	12.14d	297c	12.27f
1:0(B)	1.56b	0.14b	17.7ab	334.67b	39.01d
3:1(B:P)	1.67ab	0.15ab	18.1a	362.50ab	25.86
1:1(BP)	1.79a	0.16a	15.7c	404.00a	89.16a
1:3(BP)	1.63ab	0.13c	15.3c	361.67ab	50.00c
0:1(P)	1.55b	0.12c	15.1c	335.83b	78.96b
+AMF	1.74a	0.14a	18.1a	459.20a	88.9a
- AMF	1.58b	0.13b	15.6b	260.27b	24.57b

Total soil organic C, total N and Soil microbial biomass C in berseem and Persian clover intercrop was significantly higher ($P \le 0.001$) than in the sole crop and was more increased by AM inoculation. Available P by ratio of 3:1 berseem clover:Persian clover was the highest among treatments (Table 3). AM inoculation significantly increased P availability.

Result showed that the effect of cropping system affected N_2-fixing bacteria colony form unit (CFU) numbers (Table 3). CFU counts of N2-fixing bacteria were increased with reduced number of B in mixed cropping (1:1). There were marked differences in CFU counts of bacteria population between 0:1 and 1:0. Persian clover sole crop compared to berseem clover significantly increased ($P \leq 0.001$) N_2-fixing CFU.

The total intercropping yield from two cut gives an accurate assessment of the greater biological efficiency of the intercropping situation. Total intercropping yield values indicated that clovers recorded yield advantage in ratios 1:1 intercropping systems due to crop complementarities (Table 2). This advantage is probably due to different above- and below-ground growth habits and morphological characteristics of intercrop components causing a greater efficiency in the utilization of plant growth resources, i.e. water, nutrients and radiation energy (Fukai and Trenbath 1993; Ofori and Stern, 1987; Willey, 1979). Forage drymatter increased by micorrhizal (Table 2). The synergistic symbiosis between plants and arbescular mycorrhizal fungi has been the subjected of intensive research (Smith and Read, 1997; Hodge, 2000; Huat *et al.,* 2000; Tuffen, 2002; Yu and cheng, 2003).

In this study, there is a significant positive relationship between soil organic C and total N (Table 3). Similarly, previous studies state that if soil organic C increases, the total N increase (Manu *et al.,* 1991; Li *et al.,* 2007). The dynamics of N in mineral soil is closely linked to C, because most N exists in organic compounds and heterotrophic microbial biomass, which utilize organic C for energy. The result coincides with previous studies (Arunachalam and Arunachalam, 2002; Sharma *et al.,* 2004; Wright *et al.,* 2005). There are positive and significant relations between the soil microbial biomass C, soil organic C, total N and forage yield in PB intercrop ratio of 1:1 (Table 3). Pervious studies indicated a relations between the soil microbial biomass C, soil organic C and total N (Arunachalam and Arunachalam, 2000; Sharma et al. 2004; Wright et al. 2005; Kara and Bolat, 2008). The relatively dense structure of plants and a greater accumulation of litter and fine roots in the understorey of forest and pasture may favor the growth of microbial populations and the accumulation of C in microbial biomass (Kara and Bolat, 2008). Mixed cropping treatment led to increases microbial biomass (Table 3). Doran *et al.* (1987) found that red clover and hairy vetch significantly increased soil microbial biomass C.

Result shows that mycorrhizal plant enhanced soil microbial biomass C, soil organic C and nitrogen-fixing rhizobia (Table 3). AM fungi account for 5-50% of the biomass of soil microbes (Olsson et al., 1999). Biomass of hyphae of AM fungi may amount to 54-900 kg ha^{-1} (Zhu and Miller, 2003), and some products formed by them may account for another 3000 kg (Lovelock et al., 2004). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10-20 (Rillig *et al.,* 2001).

Result indicated AM fungi influenced microbial properties of the soil (Table 3). Van Aarle *et al.* (2003) reported an increased microbial biomass and bacterial activity in the presence of AM fungal hyphae in a limestone soil. However there is inconsistent result. For example Kim *et al.* (1998) showed that inoculation of tomato with *Glomus etunicatum* had no effects on total soil microbial biomass C.

Result indicated that mycorrhizal plant enhanced soil nitrogen-fixing rhizobia (Table 3). Colonization of plant roots by AM fungi can affect bacterial communities associated with the roots in both direct, provision of energy-rich carbon compounds derived from host assimilates, which are transported to the mycorrhizosphere via fungal hyphae, changes in pH of the mycorrhizosphere induced by the fungus, competition for nutrients, and fungal exudation of other inhibitory or stimulatory compounds and Indirect interactions include mycorrhizamediated effects on host plant growth, root exudation, and soil structure (Johansson et al., 2004). Nitrogen-fixing bacteria were higher with 1:1 treatment (Table 3). Pervious studies indicated that plant species have a strong effect on the community composition (Grayston et. al. 1998; Miethling et. al. 2000).

Result showed that mycorrhiza enhanced P pools (Table 3). It has been suggested that mycorrhizas may benefit plant growth by increasing the availability of P from non-labile sources. Cardoso *et al.* (2006) reported that while no significant changes occurred in the inorganic and organic P pools with non-mycorrhizal maize (Zea mays L.) plants, mycorrhizal plants depleted the pools of Resin-Pi and NaHCO3-Pi completely, and the pool of NaOH-Pi by about 20%. P uptake by ratio of 3:1 berseem clover:Persian clover was the highest among treatments (Table 3). Berseem shoot P uptake compared to Persian sole crop was significantly higher (data not shown). In intercropping treatment, berseem P uptake by ratio of 3:1 was the highest among treatments (data not shown). Therefore, maybe berseem fall forage returned and incorporated to the harvest area enhanced P pools.

AM plants caused to improve total N (Table 3). AM fungi may be important in nitrate uptake in mediterranean and (semi-)desert ecosystems. Because of their small size, AM fungal hyphae are better able than plant roots to penetrate decomposing organic material and are therefore better competitors for recently mineralized N (Hodge, 2003). By capturing simple organic nitrogen compounds, AM fungi can short-circuit the N-cycle (Hawkins *et al.* 2000; Hodge *et al.* 2001). However, plants seem to be better than fungi in this regard (Hodge, 2001). Finally, AM fungi can indirectly affect N availability because enhanced uptake of P is important for nodulation in legumes.

There are few reports on advantage of N uptake by clover/celear intercropping system over sole cropping. The explanation of N increase of both clovers species intercropping wasn't applicable to none legume/ none legume intercropping because that associated has no N fixation. Other factors might also influence intercropping performances such as root system dynamics, canopy structure and competitive ability towards weeds. Increasing aboveground N production of inoculation clovers with AM fungi supported by results of Barea *et al.* (1987). Azco´n-Aguilar *et al.* (1993) reported that mycorrhizal plants have access to forms of N that are unavailable to non-mycorrhizal plants.

Conclusion

In sustainable agricultural systems the resident soil microflora becomes ever more important for ecosystem processes. Results from the present research demonstrate that clover croprotation, cropping systems and AM fungal promote soil fertility such as nutrient cycling, microbial biomass C and soil nitrogen-fixing rhizobia. Different plant species and cropping systems (monoculture or mixed cropping) affect soil fertility differently dependent upon their forage quality and quantity. This study suggest that legume crop rotation compared to fallow soil may be enhanced soil fertility when compared to land fallow.

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