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Effects of mixed cropping, earthworms (*Pheretima* sp.), and arbuscular mycorrhizal fungi (*Glomus mosseae*) on plant yield, mycorrhizal colonization rate, soil microbial biomass, and nitrogenase activity of free-living rhizosphere bacteria

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Mycorrhiza;
Earthworm;
Mixed cropping;
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N-uptake

Summary

Sustainable agriculture uses nature as the model in designing agricultural systems. Because nature consistently integrates her plants and animals into a diverse landscape, a major tenet of sustainable agriculture is to create and maintain diversity. The effects of earthworm inoculation (*Pheretima* sp., Ew), arbuscular mycorrhizal fungi (*Glomus mosseae*, AMF) and mixed cropping systems on forage yield, mycorrhizal colonization rate, nitrogenase activity (NA) of free-living rhizosphere bacteria, soil microbial biomass (SMB) carbon, and the growth of clovers were studied at various mixed cropping ratios of berseem clover (*Trifolium alexandrinum* L., B) to Persian clover (*Trifolium resupinatum* L., P) (B:P = 1:0, 3:1, 1:1, and 1:3). The effects of AMF and Ew on plant yield were positive. Mixed cropping gave more stable yields than monoculture. The greatest mycorrhizal colonization rate was observed at a B:P ratio of 1:1 with a combined AM+Ew inoculant. With the AMF+Ew inoculant, the greatest NA of free-living rhizosphere bacteria was observed with B:P = 3:1. Although Ew had no significant effect on SMB, AMF increased SMB from 256 to 444 mg carbon kg^{-1}. The greatest SMB (379 mg kg^{-1}) was observed at B:P = 1:1. The greatest nitrogen accumulation in the aboveground biomass, 31.5 mg g^{-1} forage dry matter, was obtained with mixed cropping

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(B:P = 3:1) in the presence of the AMF+Ew inoculant. A combination of AMF, Ew, and mixed cropping increased the yield, mycorrhizal colonization rate, SMB, and nitrogen uptake of the clover plants, confirming the positive effects of diversity on an agriecosystem.

Introduction

Soil quality is an intricate interaction between the chemical, biological, and physical components of the soil system, involving several key factors, which are influenced by soil management practices (Suman et al., 2006). In Iran, as in other parts of the world, the soil quality on farms has been tragically diminished by intensive agriculture. This study measured potential soil biological indicators in different intercropping systems that were used to improve soil quality and the stability of crop production. Recent research has revealed that the mixed cropping system (MCS), may be significant in terms of the overall mycorrhizal colonization rate, the nitrogenase activity (NA) of free-living rhizosphere bacteria, soil microbial biomass (SMB) carbon (C), nitrogen (N) uptake, and the plant growth of different crops.

Intercropping offers farmers the opportunity to exploit nature's principle of diversity on their farms. The potential benefits of intercropping include increased yields, protein, and forage quality, greater N contribution from legumes, greater yield stability (Willey and Reddy, 1981), and a reduced incidence of pests, weeds, and diseases (Anil et al., 1998). Intercropping often gives higher cash returns and total production per hectare, relative to those of monocultures, and ensures greater resource use efficiency (Herrara and Harwood, 1974). However, farmers are unlikely to undertake the increased cost and complexity of managing mixed crops without demonstrable evidence of the potential advantages over monocropping.

Berseem clover (*Trifolium alexandrinum* L.) and Persian clover (*Trifolium resupinatum* L.) are annual leguminous forage or cover crop species, well adapted to the semiarid conditions of 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 overwinter grazing and for harvested forage in spring (Stringi et al., 1987; Martiniello, 1999). They can be grazed or cut for fodder 2–5 times a year, from late autumn to late spring in average seasons (if sown in early March), up to six times when irrigated, and twice as a summer crop (Zarea et al., 2008).

Earthworms (Ew) are an important component of the soil system, and can enhance plant growth by improving soil fertility and nutrient cycling (Lee, 1985). Earthworms can stimulate the microbial activity of the soil during its passage through their guts (Binet et al., 1998). Enhanced N mineralization is the best documented mechanism of Ew and is generally thought to be the most important. Among the mechanisms by which earthworms modify plant growth at the individual or community levels (Scheu, 2003; Brown et al., 2004), five have been suggested as responsible for the positive effects noted on plant production: (i) the increased mineralization of the soil organic matter, which increases nutrient availability (Curry and Byrne, 1992; Lavelle et al., 1992; Subler et al., 1997), especially nitrogen (N), the major limiting nutrient in terrestrial ecosystems; (ii) the modification of soil porosity and aggregation (Blanchart et al., 1999; Shipitalo and Le Bayon, 2004), which improves water and oxygen availability to plants (Doube et al., 1997; Allaire-Leung et al., 2000); (iii) the production of plant growth regulators via the stimulation of microbial activity (Nardi et al., 2002; Quaggiotti et al., 2004); (iv) the biocontrol of pests and parasites (Clapperton et al., 2001; Blouin et al., 2005); and (v) the stimulation of symbionts (Gange, 1993; Furlong et al., 2002).

Providing a direct physical link between the soil and plant roots, arbuscular mycorrhizal fungi (AMF) are important rhizospheric microorganisms. They can increase plant uptake of nutrients, especially relatively immobile elements such as P, Zn, and Cu (Tinker and Gildon, 1983), and consequently increase the root and shoot biomass and improve plant growth. The effects of AMF on the microbiological properties of the soil described in the literature are inconsistent (Hodge, 2000; Johansson et al., 2004). There are many positive (Van Aarle et al., 2003; Wamberg et al., 2003; Langley et al., 2005), negative (Lo'pez-Gutie'rrez et al., 2004; Langley et al., 2005), and no (Kim et al., 1998) interactions reported between AMF and soil microorganisms, depending on other factors such as AMF inoculum type, the plant species, the plant growth stage, the kinds of hyphae produced, and the residence time of the hyphal residues.

The hypotheses of this research were: (1) intercropping (Persian and berseem clover) increase biomass; (2) each intercrop system will differently affect SMB and the mycorrhizal colonization rate; (3) the intercrop system will affect N production; (4) AMF will affect N uptake differently in each intercrop system; (5) Ew will affect N accumulation of clover plants; (6) Ew will influence the mycorrhizal colonization rate; and (7) the performance of Persian–berseem clover intercrops will be influenced by Ew and AMF.

Materials and methods

Study area and soil properties

Field experiments were conducted at the research farm of the Seed and Plant Improvement Institute, Karaj (54°50′N, 55°35′W, and 1312 m above sea level), Iran, during the dry season (2006). The experimental sites were located in a semiarid region with a mean annual rainfall of 270 mm. The rainfall is restricted to $5\frac{1}{2}$ months a year, from November to February, with 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 °C), with a mean of 27 °C. In October, temperature falls to a minimum of 12 °C, and reaches a maximum of 48 °C in August. The soil at the site is classified as medium black, clayey, and shallow (15–20 cm in depth). The soil is characterized by low organic matter (1.08%). Across the locations, the soil pH ranges from 7.1 to 7.8, available phosphorus (P) was 12.21–13.21 mg kg^{-1}, available potassium (K) 152–287 kg ha^{-1}, cation exchange capacity 8.4–11.0 (CEC), organic matter from 1.08% to 1.017%, earthworms 2.7–3.8 worms m^{-2} at a depth of 5–35 cm, mycorrhizal spore propagules 2–3.7 kg^{-1} soil, bacteria $21.9–25.4 \times 10^5$ colony-forming units (CFU) g^{-1} soil, N_2-fixing bacteria $18.7–19.54 \times 10^3$ CFU g^{-1} soil, and *Azotobacter* $5.2–5.9 \times 10^3$ CFU g^{-1} soil. The experimental design was a randomized split-plot factorial design with four randomized complete blocks, with the main plot treatments with or without AMF (*Glomus mosseae)* inocula. The subplot treatments included five 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 (B:P), inoculated with or without Ew (*Pheretima* sp.). The clover density was 84 plants m^{-2}. Berseem and Persian clovers were hand seeded at a depth of 1.0–1.5 cm by surface broadcasting and the seed was incorporated by raking. The spring conditions were very dry, so the plots were irrigated before and after seeding. The plots were hand weeded. Our previous research (unpublished) examined the correlations between the seeding densities and plant stands in both field and greenhouse environments for 2 years. The field establishment rates for the Persian and berseem clover seeds were 40% and 70% of the seed sowing rate, respectively. The clover seeding rates were based on these data, but ultimately, the seedlings were thinned to 84 plants m^{-2} in the appropriate ratios. The plots, 3 m × 6 m in size, were separated by heavy gauge polythene barriers buried to a depth of 0.5 m into the soil, which stood (initially) 10–15 cm above the ground. The sowing date was May 18. The necessary plant protection, irrigation, and other management practices were followed during crop growth. No pesticides were applied. The clovers were grown with irrigation, given at 10-day intervals, when water to a height of 65 mm was applied. No serious incidence of insects or diseases was observed.

Earthworms

Earthworms (*Pheretima* sp.) were collected by hand from grassland, where the experiments were conducted. Ew were washed free of surface soil with distilled water and kept in a sterilized glass vessel for 24 h to minimize the number of naturally occurring AMF associated with their surfaces or gut contents. Earthworms (40 m^{-2}) of similar fresh weight (0.61 g) and length (6.2 cm) were added to the plots. The population density of earthworms were similar to that of natural populations near the field experiment.

Mycorrhizal inoculum

The mycorrhizal fungus inocula consisted of spores and hyphal root fragments from a stock culture of *G. mosseae*. The dose of inoculum (115 spores mL^{-1} of inoculum) was 60 g m^{-2}. Two years before the experiment, we used a wet-sieving technique to extract the spores. We also used the most probable number (MPN) test to determine the number of propagules (mL^{-1}) in the original sample. Because the mycorrhizal spore propagules extracted (1–2 kg^{-1}) with the wet-sieving technique and the MPN of the propagules (0.018 cm^{-3}) from the native soil were extremely low, no attempt was made to fumigate the soil. Before the experiment, the field had been sown with canola for 2 years. The AMF inoculum of *G. mosseae* was purchased from the Agricultural and Biotechnology Research Institute, Iran, and was a pure culture of *G. mosseae* isolated from Karaj (Iran). It was selected because it was commercially available in Iran and had been reported to increase the growth of some clover species. The mycorrhizal colonization rate of both clover species was assayed before the first and second cuttings. The mycorrhizal colonization assessment was performed with the method described by Brundrett et al. (1996). We sampled 100:0, 75:25, 50:50, 25:75, and 0:100 intersections per root cluster, for a total of 100 intersections per sample of BP plants from each treatment. The samples were washed in distilled water, stained with trypan blue, and the mycorrhizal colonization levels determined using the gridline intersect method of Giovanetti and Mosse (1980).

Plant sampling

Crops were harvested twice by sickle at ground level, once before corn was sown and twice before wheat was sown in this area. The first cut, regrowth harvesting (cut 2), and total aboveground biomass yields were recorded. Samples were cut from an inner plant area of 2 m^2 at 5–7.5 cm above the soil level and the clover biomasses were separated by species. Shoot samples were oven dried at 70 °C until daily checks indicated no further reduction in weight. The dried samples were weighed and allowed to remain as green manure on each plot. The dried herbage was ground, passed through a 0.5 mm screen, and analyzed for N concentration (Baruah and Barthakur, 1997).

Acetylene reduction assay

The NA of the free-living bacteria in the soil was estimated from a 100 g sample of rhizosphere soil (stuck to the roots) collected from each plant. The method of Hardy et al. (1973) was used to estimate the NA in the soils. Briefly, 100:0, 75:25, 50:50, 75:25, and 0:100 g of soil attached to the BP was placed in vessels stopped with Suba-Seals and placed in the incubation system. The incubation system was then closed tightly and 10% of the gas volume of the incubation system was replaced with acetylene. The system was incubated at temperatures ranging from 20 to 30 °C for up to 48 h. The samples were assayed for ethylene after 48 h by injecting them into a Hewlett-Packard gas chromatograph equipped with a Poropak R column (GC-148B, Shimadzu, Japan).

Microbial biomass determination

The method of Jenkinson and Powlson (1976) was used to assess the SMB after regrowth (cut 2) harvesting. Briefly, 100:0, 75:25, 50:50, and 25:75 g of soil (stuck to the roots) collected from the BP were mixed thoroughly. Then two 25 g samples from each treatment were placed in 50 mL glass beakers. One beaker was fumigated with ethanol-free chloroform in a vacuum desiccator for 24 h at room temperature in the dark. The chloroform vapor in the desiccator was removed by three successive evacuations. After the removal of the chloroform vapor by repeated evacuation, the soils were inoculated with 1% unfumigated soil and placed in 1.5 L Mason jars. This was performed 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 the soil without fumigation. The soil suspensions were filtered through Whatman no. 42 filter paper (Whatman Ltd., UK). The total organic C content in the soil extracts was measured with a dichromate oxidation method (Vance et al., 1987). The microbial biomass carbon (MBC) was calculated as the difference in extractable organic C between the fumigated and unfumigated soils, as follows:

$$\mathrm{MBC} = 2.64\,\mathrm{FEC} \qquad (1)$$

where FEC refers to the difference in extractable organic C between the fumigated and unfumigated treatments, and 2.64 is the proportionality factor of SMB released by fumigation extraction and radiation (Vance et al., 1987).

Data analysis

The analysis of variance (ANOVA) was based on the common mixed model for a split-plot factorial design. All measured variables were assumed to be normally distributed and statistical analysis by ANOVA was performed using SAS software (1990). The normal distribution of the data was determined using the Shapiro–Wilk *W* test. The significance of the differences between treatments was estimated using the LSD range test, and a main effect or interaction was deemed significant at $P \leqslant 0.05$.

Results

Mycorrhizal colonization rate and plant biomass

Clover plants were colonized after all treatments involving inoculation with AMF. Noninoculated treatments (control) had only 4–5% colonization. Ew activity did not significantly enhance the mycorrhizal colonization rate after noninoculated treatments (Figure 1). The MCS had no effect on the mycorrhizal colonization rate. Mycorrhizal colonization rates of 38%, 44.4%, 45.6%, 45.3%, and 35% were observed for ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 intersections root of the BP plants. Inoculation with AMF+MCS+Ew had an interactive effect, and the mycorrhizal colonization rate of 59.9% was greater than that for any other treatment. The B:P ratio of 1:1 had the highest mycorrhizal colonization rate in the presence of AMF inoculation and Ew (Figure 1).

At cuts 1 and 2, the berseem clover was roughly 3.5 and 1.08 times taller, respectively, than the Persian clover, regardless of the stand ratio. The average rates of plant height were 15–54 cm (cut 1) and 50–54 cm (cut 2) for Persian clover and berseem clover, respectively. At cuts 1 and 2, the berseem and Persian clover monocultures had the highest yields, and the berseem clover monoculture had the highest yield at cut 1 (Table 1). The dry matter yields of Persian clover in mixed crops were lower than the berseem clover yields in monocultures in cut 1, but the total yields of the mixtures were higher than those of the berseem clover monoculture. The total mixed cropping forage yield was highest in MCS, at 3:1 kg ha^{-1}.

At cut 2, the Persian clover monoculture had a higher yield than the berseem monoculture (Table 1). The berseem clover forage yield was also significantly ($P \leqslant 0.05$) higher in the monoculture crop (1:0) than in the mixed crops in the second cut

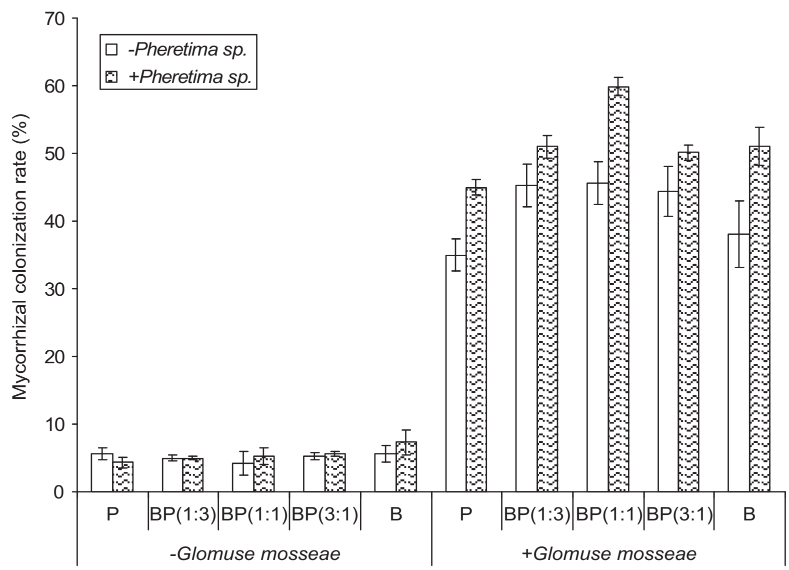

Figure 1. Mycorrhizal colonization rate (%) of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza. Means±S.E., $P<0.05$.

Table 1. Dry forage yield of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza at first cut and second cut.

Treatments		Cut 1 forage yield in monoculture and mixture (kg ha^{-1})			Cut 2 forage yield in monoculture and mixture (kg ha^{-1})		
		Berseem clover	Persian clover	Mixtures total yields	Berseem clover	Persian clover	Mixtures total yields
Cropping system							
Monoculture	B (1:0)	1574a	–	b1574	4440a	–	4440c
Mixture	B (3:1)	1301b	406a	a1701	3010b	1500a	4510c
Mixture	BP (1:1)	783.7c	691b	1474.7c	2200c	2800b	5000ab
Mixture	BP (1:3)	248d	823c	1071d	1180d	4100c	5280a
Monoculture	P (0:1)	–	988d	988d		4735d	4735b

Values within one column followed by the same liner letter are not significantly ($P \leqslant 0.05$) different; –, without mycorrhiza or earthworm; +, with mycorrhiza or earthworm.

(Table 1). The maximum dry matter yield for berseem clover under MCS were obtained for 3:1, followed by 1:1. Among the Persian clover intercrop yields, the dry matter yield of the Persian clover increased with reductions in the berseem clover plants but was significantly lower than that of the monoculture crop (Table 1). At the second cut (regrowth), the B:P ratio of 1:3 had the highest total forage intercrop yield (Table 1). There was no significant difference ($P > 0.05$) between the ratios 1:3 and 1:1.

There were no direct interactive effects between MCS, AMF, and Ew on plant yield. MCS, AMF, and Ew had significant ($P \leqslant 0.05$) positive effects on the total forage yield. Compared with the control, the combination of MCS $\times$ Ew $\times$ AM increased the shoot dry weight significantly ($P \leqslant 0.05$). The total forage yields were greater with various mixture ratios of berseem and Persian clover than those of the monocultures (Table 2).

Microbial biomass

The main treatment effects of AMF and MCS on SMB were significant. SMB was significantly higher ($P \leqslant 0.01$) after the mycorrhizal treatments than that after the no-mycorrhiza treatments. Various ratios of mixed cropping increased SMB. In contrast, Ew had no significant effect on SMB (Table 3).

Nitrogenase activity

The main effects of AM, MCS, Ew, and their interactions on NA were statistically significant ($P \leqslant 0.01$). The NA of free-living bacteria was increased by AM, Ew, and MCS at B:P = 1:1. The NA in clovers at B:P = 1:1 treated with the Ew+AM inoculant was significantly higher than that in clovers with other treatments (Figure 2).

Shoot N uptake

The main effects of AMF, Ew, and MCS treatments and their interactions on N uptake were statistically significant (Figure 3). Shoot N uptake (31.5 mg g^{-1}) after treatment with Ew and AMF at culture ratios of B:P = 1:1 was significantly higher than that after other treatments.

Table 2. Dry forage yield of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza.

Treatments	Dry forage yield (kg ha^{-1})
Arbuscular mycorrhizal fungi	
+AM	639.22a
−AM	572.34b
LSD 0.05%	23.27
B (1:0)	601.42b
B (3:1)	621.75a
Cropping system	
BP (1:1)	647.43a
BP (1:3)	635.12a
P (0:1)	572.34b
LSD 0.05%	32.53
Earthworm	
+Ew	649.04a
−Ew	577.534b
LSD 0.05%	42.92

Values within one column followed by the same liner letter are not significantly ($P \leqslant 0.05$) different; −, without mycorrhiza or earthworm; +, with mycorrhiza or earthworm.

Table 3. Soil microbial biomass carbon of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza.

Treatments	Soil microbial biomass C
Arbescular mycorrhiza	
+AM	444.53a
−AM	256.4b
LSD 0.05%	82.916
B (1:0)	327.83d
B (3:1)	357.50ab
Cropping system	
BP (1:1)	379.50a
BP (1:3)	354.58bc
P (0:1)	332.92cd
LSD 0.05%	24.53
Earthworm	
+Ew	356.4
−Ew	344.53
LSD 0.05%	15.51

Values within one column followed by the same letter are not significantly ($P \leqslant 0.01$) different; −, without mycorrhiza or earthworm; +, with mycorrhiza or earthworm.

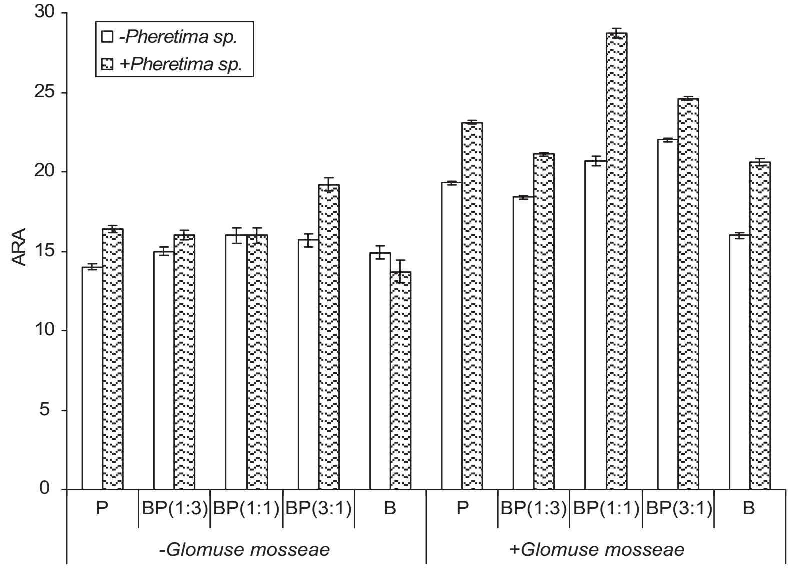

Figure 2. Free-living bacteria nitrogenase activity of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza. Means$\pm$S.E., $P<0.01$.

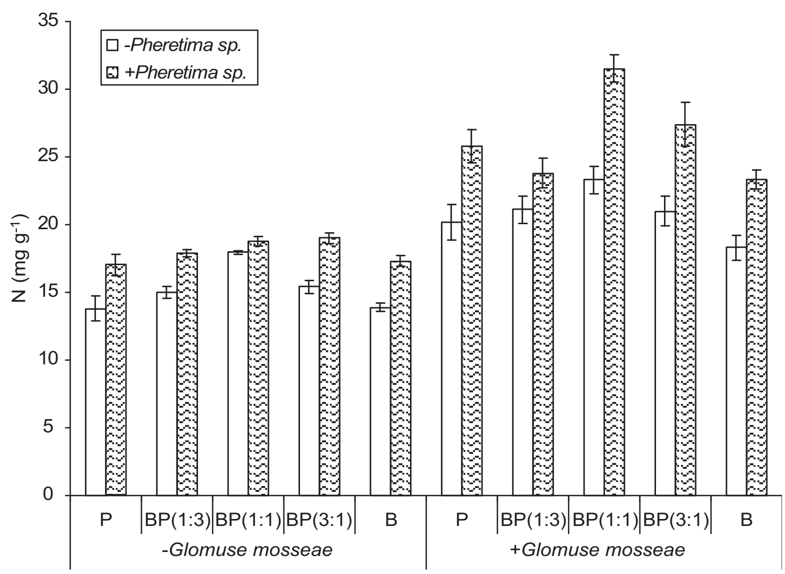

Figure 3. Shoot N-uptake (mg g^{-1}) of single or mixed cropping clovers (P and/or B) inoculated or not with earthworms and mycorrhiza. Means$\pm$S.E., $P<0.05$.

Discussion

Mycorrhizal colonization rate

Mycorrhizal colonization was higher in plants inoculated with AMF. Ew increased this positive effect on the mycorrhizal colonization rate. However, no significant differences were observed among the noninoculated treatments with and without Ew, which may be attributable to the low number of propagules in the original soil. Effective mycorrhizal root colonization is constrained by the minimal capacity of AMF for unaided dispersal through soils, but this can be enhanced by Ew (Ydrogo, 1994; Edwards and Bohlen, 1996), because Ew casts can contain up to 10 times more infective AMF propagules than occur in the surrounding soil (Gange, 1993). The enhancement of root colonization by AMF in the presence of Ew may also may attributed to the production of phytohormones by earthworms and microorganisms, which apparently stimulate mycorrhizal infection (Azcon et al., 1978). The increase in the mycorrhizal colonization rate with Ew activity was also supported by Yu and Cheng (2003). Plants inoculated with AMF at a B:P ratio of 1:1 had the greatest mycorrhizal colonization. Lebron et al. (1998) reported that the effects of Ew on mycorrhizal infections depend on species-specific interactions between earthworms and plants. With all treatments, the B:P ratio of 1:1 had the highest growth. It has been reported that AMF have a limited saprobic capacity and are dependent on plants for their carbon nutrition (Harley and Smith, 1983). Therefore, an increase in the carbon supplied by the mixed cropping B:P ratio of 1:1 may have increased the mycorrhizal colonization rate.

Plant biomass

Our results indicate the advantage of mixed crops on intercropping forage yields (cuts 1, 2, and total yield). When plant species are intercropped, it is likely that yield advantages occur as a result of the complementary use of resources by the component crops (Evans, 1960; Grimes et al., 1983; Anil et al., 1998). These results show that the productions of forage berseem clover and Persian clover monocultures were higher than that in the mixed crop cultures (Table 1). This was partly the result of the greater plant populations in the monocrops. Moreover, these crops were not subjected to interspecific competition. At the first cut, the superiority of the forage yields of berseem clover over those of Persian clover was perhaps attributable to a higher number of tillers and branches (data not shown). Despite tiller formation, the higher forage yield of the berseem clover relative to that of the Persian clover was possibly because of the more rapid accumulation of dry matter in the berseem clover. Compared with berseem clover, Persian clover generally has limited growth, especially when subjected to high temperatures. The higher temperatures during cut 1 reduced the growth rate of Persian clover compared with that of berseem clover, indicating that berseem clover better tolerates high temperatures. Furthermore, during cut 1, photosynthesis assimilation in Persian clover compared to berseem clover more served in root (data not shown) that help Persian clover regrowth.

At cut 2, the crop experienced interspecific competition. The yield of the berseem clover was affected by the Persian clover. Although the Persian clover reduced the yield of the berseem clover, an overall benefit was observed when the yields of both crops were considered together (1:3). The aboveground dry matter of the Persian clover regrowth increased more during the growth season because of a higher number of tillers and branches compared with those at cut 1 (data not shown).

The total intercropping yield (cuts 1 and 2) gives an accurate assessment of the greater biological efficiency of the intercropping system. The total intercropping yield values indicate that berseem clover recorded a yield advantage at B:P ratios of 1:3 and 1:1 in the intercropping system, attributed to crop complementarities. This advantage is probably the result of different above- and belowground growth habits and the morphological characteristics of the intercrop components, allowing their more efficient utilization of plant growth resources, i.e., water, nutrients, and radiant energy (Fukai and Trenbath, 1993). There has been no previous attempt to determine the yield of Persian/berseem clovers as a summer crop in this area, so we cannot compare this result with those of other research in the literature. However, in other areas, such as Michigan, Shrestha et al. (1998) reported a total annual forage yield of 5400 kg ha^{-1} when berseem clover was harvested in two cuttings in a year following spring seeding, and in Montana, Westcott et al. (1995) reported forage yields from a two-cutting system of 7700 kg ha^{-1}. Clovers exhibit various yields in response to climate and cropping dates. However, in another study, a ratio of Persian clover to berseem clover of 3:1 had a higher forage dry matter yield than those at other mixed cropping ratios (Zarea et al., 2008). However, in this study, there was no significant difference in the dry forage yields among the various mixed cropping ratios.

AMF fungi had a positive effect on the forage dry matter. The synergistic symbiosis between plants and AMF has been the subject of intensive research (Smith and Read, 1997; Clark and Zeto, 2000; Hodge, 2000; Huat et al., 2002; Yu and Cheng, 2003). This advantage is probably the result of the greater uptake of nutrients, especially N and P.

Our experiments have shown that the addition of Ew enhanced the clover shoot yield. This enhanced forage yield may be related to the physical and chemical improvement of the soil (Kladivko and Timmenga, 1990), the production of humic substances that can influence plant growth via physiological effects (Albuzio et al., 1986; Muscolo et al., 1993; Hu et al., 2002), or indirectly to the production of plant growth regulators via the stimulation of microbial activity by the earthworms (Nardi et al., 2002; Quaggiotti et al., 2004).

Microbial biomass

Mycorrhizal and mixed cropping treatments led to increases in SMB. An explanation for these mixed cropping effects on SMB may involve the different root growth parameters of the beseem and Persian clover plants. Previous reports by other workers have suggested root growth differences can be positive regulators of SMB (Lynch and Panting, 1980; Carter and Rennie, 1984). Mixed cropping increased the SMB, probably by inducing quantitative and qualitative rate changes in root exudation in the rhizosphere during mixed cropping compared with those of monoculture plants. When both clover species are present, different qualitative exudation rates from the root may activate different groups of microbes from those activated when one clover species is present, although this explanation cannot be confirmed here (root parameters were not measured in this study).

The increase in SMB caused by AMF may result from the increased release of exudates containing energy-rich carbon compounds derived from host plant root assimilates, host plant growth, and the soil structure (Johansson et al., 2004), which may affect SMB. AMF may also alter the SMB by causing quantitative and qualitative changes in the root exudate into the rhizospheres of the colonized plants (Hodge, 2000). Van Aarle et al. (2003) also reported an increase in microbial biomass and bacterial activity in the presence of AMF hyphae in a limestone soil. In contrast, Kim et al. (1998) showed that the inoculation of tomato plants with *G. etunicatum* had no effect on total SMB.

Nitrogenase activity

Mycorrhizal treatment led to clear increases in NA, most likely by quantitatively and qualitatively affecting the carbon and nutrients of the root exudate in the rhizospheres of infected roots (Va’zquez et al., 2000), increasing the N-fixing microorganisms (Amora-Lazcano et al., 1998) and various soil enzymes in the presence of AMF (Kim et al., 1998; Rao and Tak, 2001; Wang et al., 2006).

Mixed cropping increased the NA of free-living bacteria. The potential benefits of intercropping include increased yields, increased protein and forage quality, increased N contributions from legumes, greater yield stability, and reduced diseases (Anil et al., 1998). Intercropping berseem clover with Persian clover may affect root exudates in the rhizosphere both quantitatively and qualitatively, and in turn the activity of free-living bacteria. When both clover mixtures are cultivated together, root exudate C may increase because of the greater growth of both clovers.

Ew may increase the NA of free-living N-fixing microbes by improving the condition of the soil by modifying its porosity and aggregation (Blanchart et al., 1999; Shipitalo and Le Bayon, 2004), which improves the water and oxygen availability to plants (Doube et al., 1997; Allaire-Leung et al., 2000). Ew may also increase the release plant root exudates and create more microhabitats, which affect the diversity of microorganisms and microbial activity. Ew also stimulate microbial activity during the passage of soil through their guts (Brown et al., 2000; Tiunov et al., 2001) and increase microbial turnover, thus reducing the standing microbial stock (Bohlen and Edwards, 1995; Hendrix et al., 1998).

Shoot N uptake

When berseem clover was grown mixed with Persian clover, the total N uptake increased. The N uptake of berseem clover intercropped with Persian clover was significantly higher than that of pure crops of either clover species. The better uptake of N in the mixed system is attributed to the low levels of competition for nutrients between berseem clover and Persian clover because the duration of and variations in their rooting and shooting habits differ, especially during cut 1. The competition for resources between berseem and Persian clovers is very low, probably because Persian clover has shallower roots and a lower growth rate at cut 1 (data not shown). Although, many reports have indicated better nutrient uptake in intercropping systems, in such studies, one cropping partner is always a legume and the other a nonlegume crop. There have been few reports of an improvement in N uptake achieved with a cereal/cereal intercropping system relative to the N uptake in sole cropping. There is a close relationship between yield advantage and the nutrient uptake by the intercropped species (Morris and Garrity, 1993).

These results confirm that clover mixtures acquire large amounts of N from atmospheric fixation or the soil when in symbiosis with AMF. This is probably attributable to the more successful competition with weeds for N (data not shown) and the better use of the resources because the AMF symbiosis enhances plant growth and increases the plants’ access to forms of N that are unavailable to nonmycorrhizal plants. This corresponds to the observations of Barea et al. (1987) and Azco’n-Aguilar et al. (1993), who found greater N uptake in plants inoculated with AMF.

Ew increased the N uptake of both clovers in mixed and pure crops, which may be attributed to the increased nutrient (especially N) availability occurring with the mineralization of the soil organic matter (Curry and Byrne, 1992; Lavelle et al., 1992; Subler et al., 1997). Ew had its greatest effect on increasing the N uptake of the clovers when the berseem and Persian clovers were mixed in a ratio of 1:1 and inoculated with mycorrhiza, demonstrating the positive effects of plant diversity and organism combinations. Ew may act by enhancing the mycorrhizal symbiosis or increasing the plants' advantage in competition with weeds for N, especially when in a ratio of B:P = 1:1 (data not shown), when the N uptake of the clovers was optimal. Inoculation with either earthworms or AMF improved plant N uptake and increased plant growth. Together, Ew and AMF increased plant tissue N concentrations. Earthworm activity may increase N uptake into the roots, whereas it may not affect its transport to the aboveground plant organs, unlike AMF.

In conclusion, the combined effects of mixed cropping, AMF, and Ew are beneficial in terms of plant growth, improved N uptake, mycorrhizal colonization rate, NA of free-living N-fixing microorganisms, and SMB. These values were affected by the ratio of berseem to Persian clovers in the mixture. These results show that Persian and berseem clovers in a ratio of 1:1 produced the greatest total yield and acquired a large percentage of their N from atmospheric fixation or the soil, probably because of the low competition between the clovers and the enhanced NA. The increase in the mycorrhizal colonization rate affected by MCS and Ew was also investigated. In the presence of Ew, the mycorrhizal colonization rate was affected by the different plant mixture ratios. This experiment was performed as a field study. Under natural conditions, the relationships between organisms are extremely complex and the relationships between MCS, AMF, and Ew may have been affected by other organisms, especially when these results are compared with those of other research. For example, Yu et al. (2005) reported that Ew activity significantly increased the mycorrhizal infection rate of roots and the ryegrass shoot biomass, whereas Tuffen et al. (2002) demonstrated that AMF did not affect the growth of *Allium porrum*, although infection levels were high. Yu et al. (2005) and Azcon et al. (1978) demonstrated that the AMF infection rate of the roots was enhanced by earthworm activity; Lawrence et al. (2003) showed that earthworms reduced the colonization rates and total abundance of AMF, and altered the morphology of AM colonization in sugar maple trees (*Acer saccharum*). More investigations are required to understand the interactions between plant diversity, mycorrhiza, Ew, and free-living organisms and how they affect plant growth and carbon uptake within the life cycle of the plant under natural conditions.

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Reference

Albuzio, A., Ferrari, G., Nardi, S., 1986. Effects of humic substances on nitrate uptake and assimilation in barley seedings. Canadian Journal of Soil Science 66, 731–736.

Allaire-Leung, S.E., Gupta, S.C., Moncrief, J.F., 2000. Water and solute movement in soil as influenced by macropore characteristics. 1. Macropore continuity. Journal of Contaminant Hydrology 41, 283–301.

Amora-Lazcano, E., Vazquez, M.M., Azcon, R., 1998. Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. Biology and Fertility of Soils 27, 65–70.

Anil, L., Park, R.H.P., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass and Forage Science 53, 301–317.

Azco'n-Aguilar, C., Alba, C., Montilla, M., Barea, J.M., 1993. Isotopic (^{15}N) evidence of the use of less available N forms by VA mycorrhizas. Symbiosis 15, 39–48.

Azcon, R., Azcon-Aguilar, C., Barea, J.M., 1978. Effect of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhiza. New Phytologist 80, 359–364.

Barea, J.M., Azco'n-Aguilar, C., Azco'n, R., 1987. Vesicular-arbuscular mycorrhiza improve both symbiotic N_2 fixation and N uptake from soil as assessed with a ^{15}N technique under field conditions. New Phytologist 106, 717–725.

Baruah, T.C., Barthakur, H.P., 1997. A Textbook of Soil Analysis. Vikas Publishing House Pvt. Ltd., New Delhi, India.

Binet, F., Fayolle, L., Pussard, M., 1998. Significance of earthworms in stimulating soil microbial activity. Biology and Fertility of Soils 27, 79–84.

Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Gilot, C., Pashanasi, B., Lavelle, P., Brussaard, L., 1999. Effects of earthworms on soil structure and physical properties. In: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), Earthworm Management in Tropical Agroecosystems. CAB International, Wallingford, pp. 149–172.

Blouin, M., Zuily-Fodil, Y., Pham-Thi, A.T., Laffray, D., Reversat, G., Pando, A., Tondoh, J., Lavelle, P., 2005. Belowground organismactivities affect plant aboveground phenotype, inducing plant tolerance to parasites. Ecology Letters 8, 202–208.

Bohlen, P.J., Edwards, C.A., 1995. Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biology & Biochemistry 27, 341–348.

Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. European Journal of Soil Biology 36, 177–198.

Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant growth: burrowing into the mechanisms. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, USA, pp. 13–49.

Brundrett, M.N., Bougher, B., Dell, T., Grove, N., Malajczuk, M., 1996. Working with Mycorrhiza in Forestry and Agricultural. Australian Centre for International Agricultural Reaserch Monograph 32, Canberra, 347pp.

Carter, M.R., Rennie, D.A., 1984. Dynamics of soil microbial N under zero and shallow tillage for spring wheat, using ^{15}N urea. Plant and Soil 76, 157–164.

Clapperton, M.J., Lee, N.O., Binet, F., Conner, R.L., 2001. Earthworms indirectly reduce the effects of take-all (*Gaeumannomyces graminis* var. tritici) on soft white spring wheat (*Triticum aestivum* cv. Fielder). Soil Biology & Biochemistry 33, 1531–1538.

Clark, R.B., Zeto, S.K., 2000. Mineral acquisition by arbescular mycorrhizal plants. Journal of Plant Nutrition 23, 876–902.

Curry, J.P., Byrne, D., 1992. The role of earthworms in straw decomposition and nitrogen turnover in arable land in Ireland. Soil Biology & Biochemistry 24, 1409–1412.

Doube, B.M., Williams, P.M.L., Willmott, P.J., 1997. The influence of two species of earthworm (*Aporrectodea trapezoids* and *Aporrectoedea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. Soil Biology & Biochemistry 29, 503–509.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms, third ed. Chapman & Hall, London, 426pp.

Evans, A.C., 1960. Studies of intercropping maize or Sorghum with groundnut. East African Agricultural and Forestry Journal 26, 1–10.

Fukai, S., Trenbath, B.R., 1993. Processes determining intercrop productivity and yields of component crops. Field Crops Research 34, 247–271.

Furlong, M.A., Singleton, D.R., Coleman, D.C., Whitman, W.B., 2002. Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and casts of the earthworm *Lumbricus rubellus*. Applied and Environmental Microbiology 68, 1265–1279.

Gange, A.C., 1993. Translocation of mycorrhizal fungi by earthworms during early succession. Soil Biology & Biochemistry 25, 1021–1026.

Giovanetti, H.W., Mosse, B., 1980. An evaluation techniques for measuring vesicular-arbescular mycorrhiza infection in roots. New Phytologist 84, 489–500.

Grimes, A., Quasem, A., Sabajal Uddin, M., Jahiruddin, M., Mallik R.N., 1983. The Performance of Different Cropping Patterns in 1992–1993 at the Cropping System Research Site, Hathazari, ctg RARS. Publication no. 1.

Hardy, R.W., Burns, R.C., Holsten, R.D., 1973. Application of acetylene–ethylene assay for measurement of nitrogen fixation. Soil Biology & Biochemistry 5, 47–81.

Harley, J.L., Smith, S.E., 1983. Mycorrhizal Symbiosis. Academic Press, London.

Hendrix, P.F., Peterson, A.C., Beare, M.H., Coleman, D.C., 1998. Longterm effects of earthworms on microbial biomass nitrogen in coarse and fine textured soils. Applied Soil Ecology 9, 375–380.

Herrara, W.A.T., Harwood, R.R., 1974. The effect of plant density and row arrangement on productivity of corn rice intercrop. Paper presented at the 5th Annual Convention of the Crop Science Society of Philippines. Nagar City, May, pp. 16–18.

Hodge, A., 2000. Microbial ecology of the arbuscular mycorrhiza. FEMS Microbiology Ecology 32, 91–96.

Hu, P., Liu, D.H., Hu, F., Shen, Q.R., 2002. Plant hormones in earthworm casts and their promotion on adventitious root formation of mung bean cutting. Acta Ecologica Sinica 22, 1211–1214.

Huat, O.K., Awing, K., Hashim, A., Majid, N.M., 2002. Effects of fertilizers and vesicular-arbucular mycorrhizas on the growth and photosynthesis of *Azadirachta excelsa* (jack) jacobs seedling. Forest Ecology and Management 158, 51–58.

Jenkinson, D.S., Powlson, D.S., 1976. The effect of biological treatments of metabolism in soil. V.A method for measuring soil biomass. Soil Biology & Biochemistry 8, 209–213.

Johansson, J.F., Paul, L.R., Finlay, R.D., 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiology Ecology 48, 1–13.

Kim, K.Y., Jordan, D., McDonald, G.A., 1998. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biology and Fertility of Soils 26, 79–87.

Kladivko, E.J., Timmenga, H.J., 1990. Earthworms and agricultural management. In: Box, J.E., Hammond, L.C. (Eds.), Plant Hormones and their Role in Plant Growth and Development. Martinus Nijhoff, Dordrecht, pp. 593–613.

Knight, W.E., 1985. Miscellaneous annual clovers. In: Taylor, N.L. (Ed.), Clover Science and Technology. Agronomy Monograph 25. ASA, CSSA, and SSSA, Madison, WI, pp. 547–551.

Langley, J.A., Johnson, N.C., Koch, G.W., 2005. Mycorrhizal status influences the rate but not the temperature sensitivity of soil respiration. Plant and Soil 277, 335–344.

Lavelle, P., Melendez, G., Pashanasi, B., Schaefer, R., 1992. Nitrogen mineralization and reorganization in casts of the geophagous tropical earthworm

Pontoscolex corethrurus (Glossoscolecidae). Biology and Fertility of Soils 14, 49–53.
Lawrence, B., Melany, C.F., Timothy, J.F., Suárez, E.R., 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytologist 157, 145–153.
Lebron, L., Zou, X.M., Lodge, D.J., 1998. Disturbance VA Mycorrhizae by Earthworm in a Pasture and a Forest in Puerto Rico. Second International Conference on Mycorrhiza, Uppsala, Sweden, pp. 5–10.
Lee, K.E., 1985. Earthworms: their Ecology and Relationships with Soils and Land Use. CSIRO, Sydney.
Lo'pez-Gutie'rrez, J.C., Toro, M., Lo'pez-Herna'ndez, D., 2004. Arbuscular mycorrhiza and enzymatic activities in the rhizosphere of *Trachypogon plumosus* Ness. In three acid savanna soils. Agriculture, Ecosystems & Environment 103, 405–411.
Lynch, J.M., Panting, L.M., 1980. Cultivation and the soil biomass: N (and presumably, SMB) in root-excluded, inter-rows. Soil Biology & Biochemistry 12, 29–33.
Martiniello, P., 1999. Effects of irrigation and harvest management on dry matter yield and seed yield of annual clover grown in pure stand and in mixtures with graminaceous species in a Mediterranean environment. Grass and Forage Science 54, 52–61.
Morris, R.A., Garrity, D.A., 1993. Resource capture and utilization in intercropping: non-nitrogen nutrients. Field Crop Research 34, 319–334.
Muscolo, A., Felici, M., Concheri, G., Nardi, S., 1993. Effect of earthworm humic substances on esterase and peroxidase activity during growth of leaf explants of *Nicotiana plumbginifolia*. Biology and Fertility of Soils 15, 127–131.
Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biology & Biochemistry 34, 1527–1536.
Quaggiotti, S., Ruperti, B., Pizzeghello, D., Francioso, O., Tugnoli, V., Nardi, S., 2004. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). Journal of Experimental Botany 55, 803–813.
Rao, A.V., Tak, R., 2001. Influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoil in India. Applied Soil Ecology 17, 279–284.
SAS, 1990. SAS Procedure Guide, Version 6, third ed., SAS Institute, Cary, NC, 705pp.
Scheu, S., 2003. Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia 47, 846–856.
Shipitalo, M.J., Le Bayon, R.C., 2004. Quantifying the effects of earthworms on soil aggregation and porosity. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, 441pp.
Shrestha, A., Hesterman, O.B., Squire, J.M., Fisk, J.W., Sheaffer, C.C., 1998. Annual medics and berseem clover as emergency forages. Agronomy Journal 90, 197–201.
Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis. Academic Press, San Diego, 605pp.
Stringi, L., Amato, G., Gristina, L., 1987. Trifoglio alessandrino in ambiente semi-arido: influenza dello stadio di utilizzazione e della dose di seme sulla produzione di foraggio e di seme. L'Informatore Agrario 26, 63–68.
Subler, S., Baranski, C.M., Edwards, C.A., 1997. Earthworm additions increased short-term nitrogen availability and leaching in two grain crop agroecosystems. Soil Biology & Biochemistry 29, 413–421.
Suman, A., Lal, M., Singh, A.K., Gaur, A., 2006. Microbial biomass turnover in Indian subtropical soils under different sugarcane intercropping systems. Agronomy Journal 98, 698–704.
Tinker, P.B., Gildon, A., 1983. Mycorrhizal fungi and ion uptake. In: Robb, D.A., Pierpoint, W.S. (Eds.), Metals and Micronutrients. Uptake and Utilization of Metals by Plants. Academic Press, London, pp. 21–32.
Tiunov, A.V., Bonkowski, M., Alphei, J., Scheu, S., 2001. Microflora, Protozoa and Nematoda in *Lumbricus terrestris* burrow walls: a laboratory experiment. Pedobiologia 45, 46–60.
Tuffen, F., Eason, W.R., Scullion, J., 2002. The effect of earthworm and arbuscular mycorrhizal fungi on growth of and ^{32}P transfer between *Allium porrum* plants. Soil Biology Biochemistry 34, 1027–1036.
Van Aarle, I.M., Söderström, B., Olsson, P.A., 2003. Growth and interactions of arbuscular mycorrhizal fungi in soils from limestone and acid rock habitats. Soil Biology & Biochemistry 35, 1557–1564.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.
Va'zquez, M.M., Ce'sar, S., Azco'n, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology 15, 261–272.
Wamberg, C., Christensen, S., Jakobsen, I., Müller, A.K., Sørensen, S.J., 2003. The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biology & Biochemistry 35, 1349–1357.
Wang, F.Y., Lin, X.G., Yin, R., Wu, L.H., 2006. Effects of arbuscular mycorrhizal inoculation on the growth of *Elsholtzia splendens* and *Zea mays* and the activities of phosphatase and urease in a multi-metal-contaminated soil under unsterilized conditions. Applied Soil Ecology 31, 110–119.
Westcott, M.P., Welty, L.E., Knox, M.L., Prestbye, L.S., 1995. Managing alfalfa and berseem clover for forage and plowdown nitrogen in barely rotations. Agronomy Journal 87, 1176–1181.
Willey, R.W., Reddy, M.S., 1981. A field technique for Abul Hossain, M., effect of intercropping groundnut with onion at varying planting arrangement. Bangladesh Journal of Agricultural Research 22, 23–30.

Ydrogo, H.F.B., 1994. Effecto de las lombrices de tierra (*Pontoscolex corethrurus*) en las micorrizas vesiculo arbusculares (M.V.A.) en la etapa de crecimento de araza (*Eugenia stipitata*), achiote (*Bixa orellana*), pijuayo (*Bactris gasipaes*), en suelos ultisoles de Yurimaguas. Thesis, University Nac. San Martin, Peru.

Yu, X.Z., Cheng, J.M., 2003. Effect of earthworm on bioavailability of Cu and Cd in soils (in Chinese). Acta Ecologica Science 23 (5), 922–992.

Yu, X., Cheng, J., Wong, H.M., 2005. Erthworm_mycorrhiza interaction on Cd uptake and growth of ryegrass. Soil Biology & Biochemestry 37, 195–201.

Zarea, M.J., Ghalavand, A., Mohammadi Goltapeh, E., Rejali, F., 2008. Influence of forage legumes mixed cropping on biomass yield, soil microbial biomass and nitrogenase activity. Green Farming Journal 1 (6), 12–15.