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Different farming and water regimes in Italian rice fields affect arbuscular mycorrhizal fungal soil communities

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Abstract. Arbuscular mycorrhizal fungi (AMF) comprise one of the main components of soil microbiota in most agroecosystems. These obligate mutualistic symbionts colonize the roots of most plants, including crop plants. Many papers have indicated that different crop management practices could affect AMF communities and their root colonization. However, there is little knowledge available on the influence of conventional and low-input agriculture on root colonization and AMF molecular diversity in rice fields. Two different agroecosystems (continuous conventional high-input rice monocropping and organic farming with a five-year crop rotation) and two different water management regimes have been considered in this study. Both morphological and molecular analyses were performed. The soil mycorrhizal potential, estimated using clover trap cultures, was high and similar in the two agroecosystems. The diversity of the AMF community in the soil, calculated by means of PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and 18S rDNA sequencing on clover trap cultures roots, was higher for the organic cultivation. The rice roots cultivated in the conventional agrosystem or under permanent flooding showed no AMF colonization, while the rice plants grown under the organic agriculture system showed typical mycorrhization patterns. Considered together, our data suggest that a high-input cropping system and conventional flooding depress AMF colonization in rice roots and that organic managements could help maintain a higher diversity of AMF communities in soil.

Key words: arbuscular mycorrhizal fungi (AMF); conventional farming systems; fungal soil communities; organic farming systems; rice.

Introduction

Italy is the largest rice producer in the European Union and one of the major non-Asian producers, after Brazil, the United States, and Egypt. Rice cultivation in Italy is mostly located in the northern regions and extends over ~224 000 ha, which represent 1.4% of the total arable area (Ente Nazionale Risi, statistics 2008; *available online*).[6] The north of Italy offers environmental conditions that, although not ideal, are nevertheless suitable for the cultivation of rice, which requires hot summers and enormous quantities of water. The areas dedicated to rice farming are basically concentrated between Piedmont and Lombardy, where the channeling of natural watercourses to periodically flood the rice fields is particularly simple. Rice cultivation in this productive area has reached a peak thanks to modern agricultural systems, but the conventional way of cultivating rice becomes more and more challenging due to adverse abiotic stress conditions, such as water shortage, along with environmental deterioration caused by a high input of fertilizers and pesticides (Ghosh and Bhat 1998, Ueji and Inao 2001, Choudhury et al. 2007, Toshisuke et al. 2008). Therefore, the development of alternative solutions, while taking into account the current environmental conditions and available resources, is crucial to increase rice production. In recent years, low-input agricultural systems have gained increasing importance in many industrialized countries since they involve the conservation of natural resources and a reduction in environmental degradation (Mäder et al. 2002). Conventional farming systems with a reduced input of fertilizers and pesticides have been developed, but more and more farmers have also changed to low-input, organic farming systems where fertilization is mainly, or exclusively, based on on-farm-produced animal manure, in addition to the nutrient input received from legume-based crop rotations and green manure. This management practice often leads to negative nutrient balances and a reduced nutrient availability (Oehl et al. 2002). It is under these conditions that plants are expected to be particularly dependent on an effective symbiosis with arbuscular mycorrhizal fungi (AMF;

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[6] ⟨http://www.enterisi.it/ser_statistiche.jsp⟩

Scullion et al. 1998, Smith and Read 2008). AMF are believed to support plant growth by increasing the supply of immobile soil nutrients, notably P, enhancing tolerance or resistance to soil pathogens and abiotic stresses, and by improving the soil structure (Helgason and Fitter 2009). These beneficial effects of AMF are important in natural ecosystems, although they may be less crucial in high-input agriculture (Barea and Jeffries 1995, Galvez et al. 2001, Galvan et al. 2009). Clearly, agricultural management factors such as the intensity of cultivation, the quality and quantity of applied fertilizers, and the plant protection strategies, may have severe impacts on the AMF community structure (Sieverding 1989, Douds and Millner 1999, Oehl et al. 2003). Reports on AMF communities retrieved in farming systems with different management practices, such as tillage, fertilizer input, pesticide use, and crop rotation, were reviewed by Douds and Millner (1999). According to Gosling et al. (2006) AMF can be used in agriculture to increase crop yields while minimizing the requirement of chemical fertilizers. In spite of numerous studies claiming substantial yield increases, mycorrhizal technology is still far from being routinely applied in agricultural practices.

Many works describing the symbiosis between AMF and rice can be found in the literature. As rice is a model plant, a great number of data on molecular, biochemical, and physiological studies on AMF symbiosis can be found. However, very few works deal with the study of AMF biodiversity in rice field conditions, on the influence of cropping systems on the establishment of the symbiosis, and on the effect of the symbiosis on the rice plant growth. Moreover, the data that can be found are mainly derived from pot culture experiments.

The present work is a snapshot on the biodiversity of AMF in the rice rhizosphere in relation to agricultural practices. It investigates and compares the arbuscular mycorrhizal fungal communities in wetland rice fields in conventional and low-input agroecosystems, under different nutrient and water management conditions.

Materials and Methods

Sampling sites

The investigation was carried out in two adjacent farms in Pavia, Italy (Appendix). The two farms adopt two different kinds of crop management practices: conventional farming (geographical position 45°12′55.13″ N, 9°7′42.23″ E) with continuous high-input rice monocropping and organic farming (geographical position 45°12′18.91″ N, 9°7′11.39″ E) with a five-year crop rotation (rice–rice–soybean–corn–winter cereal [barley or spelt]). No cover crops or weeds were present in the conventional rice fields over the winter. This pairing of neighboring organic and conventional fields, which are ~1300 m far apart, was chosen to ensure that the climate and soil conditions were approximately the same. The climate of the region is temperate, with 465 mm of rainfall and an average temperature of 13.5°C during the year and of 17.60°C during the crop season (March–October). Soil is classified as a Typic Hapludalf Haplic Luvisols (FAO), coarse-loamy, mixed, and mesic in both farms. Table 1 summarizes the soil properties. The organic carbon (OC) in the continuous rice production is higher because of the submerged condition, while it is lower in the organic fields because the dry condition (during three out of the five-year crop rotation program) leads to a faster OC turnover (Sahrawat 2003).

Table 1. Chemical and physical characteristics of the soil used in the experiment.

Variable	Conventional farming	Organic farming
pH (H_2O)	6.35	5.74
Electrical conductivity (1:2, μS/cm)	164	62
Total organic C (%)	1.69	0.89
Total N (g/kg)	1.63	0.90
Available P_2O_5 (ppm)	272	161
Exchangeable K (ppm)	122	80
Exchangeable Na (ppm)	24	10
Exchangeable Ca (ppm)	1379	686
Exchangeable Mg (ppm)	143	95
Clay (%)	1.95	3.45
Silt (%)	21.00	17.45
Sand (%)	77.05	79.10

The soils and rice plants (*Oryza sativa* L. cv. Baldo) were sampled in 2003 and 2004. In 2003, the sampling was conducted in a 24 300-m^2 (215 × 113) rice field in the conventional farm and in a 45 000-m^2 (276 × 166) rice field in the organic farm. Dry seeding was performed and flooding occurred at tillering stage, in both fields (Table 2). The organic rice was at the second year of the crop rotation program. In the conventional farm, the fertilization consisted of a 12.5.20 N:P:K mineral complex (375 kg/ha) applied before seeding and DAP (Diammonium phosphate; 150 kg/ha) applied before tillering. The field was subjected to pendimetalin and oxadiazon herbicide treatments at pre-emergence and propanil and etoxysulfuron at post-emergence. N and P fertilizations, consisting of cattle manure + poultry litter + horn and hoofs (1500 + 300 + 420 kg/ha, respectively) and K-Mg sulphate (270 kg/ha) were applied in the organic farm. The actual amounts of the nutrients applied in the different fields are shown in Table 3. No herbicides were used. Samples were collected just before flooding (June 2003).

In 2004, the sampling was carried out in three fields only in the organic farm. Dry seeding was performed in all the fields, and flooding occurred on two different occasions. Three conditions were considered: organic N and P fertilization + conventional flooding (at tillering stage in June), organic N and P fertilization + delayed flooding (in August), no N and P fertilization + delayed flooding (in August). The organic N and P fertilization consisted of poultry litter + horn and hoofs (1200 + 330 kg/ha). All the fields also received K-Mg sulphate (230 kg/ha). The actual amounts of the nutrients applied in the different fields are shown in

TABLE 2. Field operations in 2003.

Operation	Conventional farming	Organic farming
Management	Continuous high-input rice monocropping	5-year crop rotation
Fertilization	N:P:K mineral complex	Organic N and P + sulfate K-Mg
Seeding	2 May 2003	25 May 2003
Flooding	11 June 2003	17 June 2003
Sampling	10 June 2003	16 June 2003

Table 3. No herbicides were used. Samples were collected twice, in June and August, just before the two flooding events (Table 4).

Soil and plant sampling

Five equidistant square areas (1.5 m of side) were chosen in each field, on each of the two diagonals, for a total of nine sampling squares (Appendix). Five plants were collected in each sampling square (one for each corner and one in the center). Each plant was collected by removing a block of soil of ~15 cm diameter and depth, containing plant roots. The plants were kept separate, while the soils from each square were combined; therefore, a total of 9 soil cores and 45 rice plants were obtained for each field. The samples were stored in polyethylene bags before transporting them to the laboratory, where the plant roots were carefully washed free of soil particles and organic debris under tap water, quickly dried on paper, and then used for morphological and molecular analyses.

AM fungal trap culture

The soils sampled in 2003 in the two rice agroecosystems were used as inocula to establish trap cultures with *Trifolium repens*. One trap culture pot was established for each soil core, for a total of nine pots for the biological farming system and nine for the conventional one. The bottom of each pot was covered with a drainage mat and filled with 650 g of autoclaved sand. About 100 g of soil inoculum were placed on the surface of the sand in the pots, and then covered with 250 g of autoclaved sand. *T. repens* seeds were randomly planted in each pot. The trap cultures were kept in a growth chamber with a photoperiod of 16 h of light and 8 h of darkness at 23°C and 21°C, and irrigated once a week with a low-phosphate Long Ashton solution (Hewitt 1966) and twice a week with tap water. After four months, the clover plants were collected and the roots were used to evaluate the mycorrhizal potential of the two sites as well as AMF biodiversity.

Morphological evaluation of AMF root colonization

The roots from the field rice and from the trap culture clover were stained with 0.1% cotton blue in lactic acid. One-centimeter root fragments were then mounted onto microscopy slides and the intensity of the root cortex colonization by AM fungi and the presence of arbuscules were determined, as described by Trouvelot et al. (1986). The results were statistically analyzed by one way ANOVA, with Tukey as the post hoc test, using the Systat version 10 program (Systat Software 2000). Differences were considered significant when the P value was <0.05.

DNA extraction and PCR

Root fragments (1–2 cm each) of the 45 rice plants from each field or of clover plants from the nine different soil cores were pooled for DNA preparations. Total DNA was extracted from the pools of the root fragments according to the protocols described in Vallino et al. (2006). The DNA was resuspended in 20 μL of sterilized water. Several dilutions of extracted DNA (1/10, 1/50, 1/250) were prepared.

Partial ribosomal small subunit (SSU) DNA fragments were amplified using 0.7 units of Red*Taq* DNA polymerase (Sigma) and two different sets of primers. One set consisted of a universal eukaryotic primer NS31 (Simon et al. 1992) and the AM1 primer, designed to

TABLE 3. Actual amount of nutrients applied in the different fields.

Operation	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
Conventional farming 2003			
Pre-seeding	45	19	75
Tillering	27	69	
Total before sampling	72	88	75
Organic farming 2003			
Pre-seeding	122	55	39
Organic farming 2004 field 1 and 2			
Pre-seeding	82	36	29
Organic farming 2004 field 3			
Pre-seeding	0	0	29

TABLE 4. Field operations in 2004, organic farming only.

Operation	Field 1	Field 2	Field 3
Fertilization	Organic N and P + K-Mg sulphate	Organic N and P + K-Mg sulphate	K-Mg sulphate
Seeding	21 May	28 May 2004	28 May 2004
Flooding	20 June	13 August	13 August
Sampling 1	18 June	18 June	18 June
Sampling 2	12 August	12 August	12 August

amplify AM fungal SSU sequences, but not plant sequences (Helgason et al. 1998). The second polymerase chain reaction (PCR) assay targeted the *Glomeromycota* lineages, whose 18S rDNA sequences are too divergent to be amplified with the aforementioned primer set. The PCR reaction was then conducted with the specific primer ARCH1311 (Redecker 2000) in combination with NS8 (White et al. 1990). PCR reactions were performed using 0.1 μg/μL BSA, 0.2 mM dNTPs, 0.5 μM of each primer, and the supplied reaction buffer to a final volume of 25 μL (PCR conditions: 95°C for 3 min, then 35 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, and then 72°C for 10 min).

Cloning, RFLP typing, and sequencing

The PCR products were purified following the QIAquick protocol (Qiagen, Milan, Italy). Cloning was done using the pGEM-T vector system (Promega, Milan, Italy) and transformed into *Escherichia coli* (Xl1 blue). Putative positive transformants were screened in each resulting SSU rRNA gene library, using a second NS31/AM1 or ARCH1311/NS8 amplification with the same conditions described in the previous section. Positive clones from each sample were tested for restriction fragment length polymorphism (RFLP) by independent digestion with *Hinf*I and *Hs*p92II for the set of NS31/AM1 primers or with *Hinf*I and *AluI* for ARCH1311/NS8, according to the manufacturer's instructions (Promega), and analyzed on 3% agarose (in Tris/Borate/EDTA; TBE) gel electrophoresis.

Examples of each RFLP type were sequenced using the universal primers SP6 and T7. Sequence editing was done using the Sequencher program version 4.1.4 (Gene Codes Corporation 2006). The sequences have been deposited at the National Center for Biotechnology Information (NCBI) GenBank (*available online*)[7] under accession numbers HM051287-HM051334.

Diversity analysis

Rarefaction curves were obtained using the free Analytical Rarefaction Program software version 1.3 (*available online*)[8] by plotting the number of observed RFLP profiles against the number of analyzed clones.

The Shannon-Weaver (H') index was calculated on the RFLP data as an additional measure of AMF diversity, since it combines two diversity components, i.e., species richness and evenness. The following formula was used:

$$H' = -\sum_{j=1}^{s} p_j \log p_j.$$

Phylogenetical analysis

Sequence similarities were determined using the BLASTn sequence similarity search tool (Altschul et al. 1997) provided by GenBank. Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from Genbank. ClustalX (Thompson et al. 1997) was used for multiple alignments, which were manually adjusted in GeneDoc (Nicholas and Nicholas 1997). We ran neighbor-joining (NJ) and maximum parsimony (MP) phylogenetic analyses with the PAUP4.08b program (Swofford 2002) using the default parameters. *Chytridiomycota Blastocladiella emersonii* was chosen as the outgroup and used to root the trees. Sequences chosen to represent the currently known genera from *Glomeromycota* were included in the phylogenetic tree as references. Where appropriate, preference was given to similar database entries that were found in the BLAST searches.

RESULTS

AMF colonization of rice roots in different conditions

The degree of root colonization by AM fungi was determined microscopically after cotton blue staining (Table 5). Mycorrhizal colonization was absent in all the sampled plots in conventional treatment, while it ranged from 0% to 34% root length in the organic management system. The degree of colonization observed in June in both years was comparable (2.45%, 5.71%, 3.40%) in the plants grown under organic N and P fertilization. The plants grown without N and P fertilization instead had a greater presence of AMF (25%). The fungus then disappeared after two months of flooding. Without flooding, fungal colonization instead increased (34%) under N and P fertilization or remained the same (18%) in the absence of N and P fertilization. Where present, the fungus formed the typical structures, i.e., intercellular and intracellular hyphae and arbuscules.

[7] ⟨http://www.ncbi.nlm.nih.gov⟩
[8] ⟨http://www.uga.edu/~strata/software/Software.html⟩

TABLE 5. Rice root colonization under different conditions.

Agricultural system	N and P fertilization	Herbicide applications	Flooding	Sampling	F%
Conventional	mineral	yes	June	June 2003	0.00
Organic	organic	no	June	June 2003	2.51[a]
Organic	organic	no	June	June 2004	5.71[a]
Organic	organic	no	delayed (August)	June 2004	3.40[a]
Organic	no	no	delayed (August)	June 2004	25.00[b]
Organic	organic	no	June	August 2004	0.00
Organic	organic	no	delayed (August)	August 2004	34.00[c]
Organic	no	no	delayed (August)	August 2004	18.00[b]

Notes: F% is the intensity of root cortex colonization by AM fungi. Means in each column followed by the same letter do not differ statistically ($P < 0.05$).

AMF infection potential of the soil

In addition to the direct analysis on the field rice roots, the clover trap cultures roots were morphologically analyzed to evaluate the mycorrhizal potential in the soils sampled in 2003 in the two agroecosystems. The mycorrhizal potential was high and not significantly different in the conventional and organic agricultural systems: in fact all the analyzed root fragments presented AM fungal colonization (100%), with a presence of arbuscules of 21.62% and 26.56%, respectively.

Molecular detection and diversity of the AM fungi

The DNA extracted from the field rice roots failed to give amplification in the PCR reactions, probably due to the very low AMF colonization rate. However, the DNA extracted from the clover trap culture roots was successfully amplified with both primer pairs (AM1/NS31 and ARCH1311/NS8), and gave the expected bands of about 550 and 500 bp (base pairs), respectively.

As for the AM1/NS31 fragments, 53 clones from the conventional system and 65 from the organic system were subjected to RFLP analysis with the two restriction enzymes, *Hinf*I and *Hsp92*II, and 12 different patterns were produced (Fig. 1A). Four of these patterns (RFLP1, RFLP3, RFLP5, RFLP8) were present in both the conventional and organic agroecosystems. RFLP1 represented 58.5% and 55.4% of the analyzed clones, respectively. RFLP5 occurred in both agroecosystems with an average of 13.2% and 12.3%, respectively, while the other RFLPs contributed with less than 10% of the clones. The remaining patterns seemed specific for each cultivation systems: RFLP2 (9.2%), RFLP9 (9.2%), RFLP10 (1.5%), RFLP11 (3.1%), RFLP12 (1.5%) for the organic and RFLP4 (17.0%), RFLP6 (3.8%) and RFLP7 (1.9%) for the conventional ones. One to seven clones were sequenced for each RFLP type for a total of 37 sequences; the other 81 sequences were classified by means of RFLP typing. BLASTn searches in the GenBank database showed that about 11% of the clones in both agroecosystems belonged to fungal contaminants that were unspecifically amplified by the NS31/AM1 primers pairs.

As for the ARCH1311/NS8 amplification, 27 clones from the conventional system and 35 from the organic system were subjected to RFLP analysis with the two *Hinf*I and *Alu*I restriction enzymes and produced four different patterns (Fig. 1B). The most dominant RFLPs, in both agroecosystems, were RFLPa, with a percentage of 63.0% and 60.0%, and RFLPb, with a percentage of 37.0% and 31.4% of the analyzed clones. RFLPc and RFLPd were only associated with organic farming. One to five clones were sequenced for each RFLP type for a total of 11 sequences; the other 51 sequences were classified by means of RFLP typing. BLASTn searches in the GenBank database showed that all the analyzed sequences belong to *Paraglomerales*/*Archaeosporales*.

Rarefaction curves were constructed to determine whether the number of clones was sufficient to represent *Glomeromycota* diversity (Fig. 2). All the rarefaction curves almost reached a plateau, and the standard deviation range decreased as the sample size increased. These data indicated that the number of analyzed clones provided a reasonable coverage of the AM fungal diversity. The number of expected RFLP types was higher in the organic sample than in the conventional one. This observation is consistent with a higher Shannon-Weaver index in the organic system ($H' = 1.79$) than in the conventional one ($H' = 1.08$).

Identification of AM fungal groups

The BLASTn searches in the GenBank database showed a high similarity (95–100% identity) to members of the *Glomeromycota* phylum. Alignment of the sequences obtained in this study with those corresponding to the closest matches from GenBank produced trees which revealed that the sequences belong to the *Glomerales*, *Archaeosporales* or *Paraglomerales* groups.

The phylogenetic relationships among the AM1/NS31 sequences clearly revealed discrete sequence groups and allowed us to identify 10 potentially taxonomic units or fungal types (named Glo1 to Glo10), all belonging to *Glomeraceae*. Each group was supported by a bootstrap $> 75\%$ and the sequence identity within the clusters ranged from 95% to 99% (Fig. 3). The taxonomic groups were consistent with the RFLP types, with some exceptions. Group Glo8 included a sequence with a

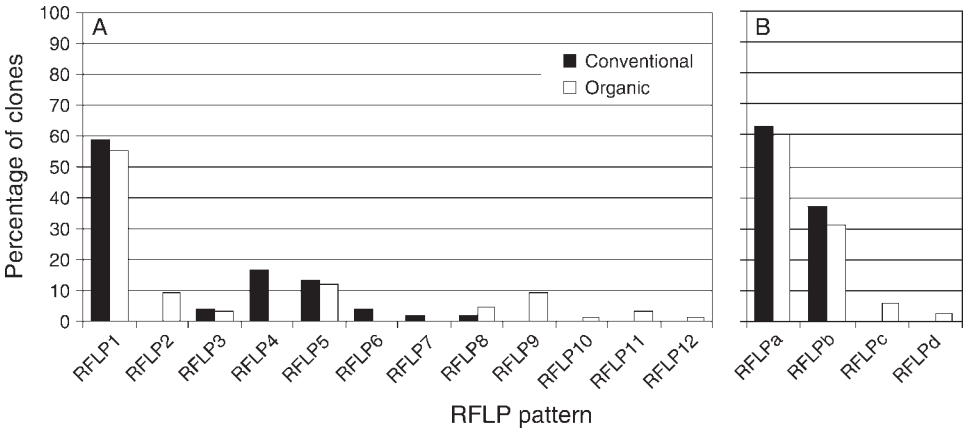

FIG. 1. Distribution and frequencies within arbuscular mycorrhizal clones in the two agricultural systems (conventional vs. organic) of the RFLP (restriction fragment length polymorphism) patterns obtained (A) after the *Hinf*I and *Hsp*92II digestions of the 118 NS31/AM1 amplified fragments and (B) after the *Hinf*I and *Alu*I digestions of the 62 ARCH1311/NS8 amplified fragments. The rDNA arbuscular mycorrhizal fungi (AMF) amplified fragments were obtained from clover trap culture roots.

99% identity to the *Glomus luteum* SA101–1 sequence. Three different subgroups were distinguished in the Glo1 phylotype: Glo1a, Glo1b and Glo1c. The Glo1 clade includes sequences with 99% identity to *Glomus mosseae* BEG69, BEG122, AFTOL-ID isolates. The sequences in phylotype Glo9 were not closely related to any current database entries and showed a low percentage of the clones found (<4%). Clade Glo10 included sequences with over 99% identity to the sequences for *G. intraradices* (AJ301859). None of the other AM fungal types clustered closely with any database sequence from the AMF isolates maintained in culture, whose identity is certain.

Four phylotypes were detected by means of phylogenetic analysis of the representative sequences amplified using the ARCH1311/NS8 primer pair (Fig. 4). These were named Para 1, Para 2, Arch 1 and Arch 2 because the sequences groups clearly fall into one of the two families, Paraglomeraceae or Archaeoporaceae, respectively. In general, the sequences that shared the same RFLP pattern did not correspond to a well defined phylotype. Para2 included sequences that were closely related to a sequence of *Paraglomus brasilianum* AJ301862. Para1 did not cluster with any known sequences of the database and formed a distinct clade with a bootstap value >80%. The Arch1 phylotype clustered with the AF452634 sequence belonging to *Archaespora* sp. while the Arch2 phylotype sequence clustered with a known sequence, Y17634, of *Archaeospora trappei* (sequence identity 95%).

DISCUSSION

Arbuscular mycorrhizal fungi possess important attributes that make them potential players in the agriculture of the future. Some agricultural practices, such as excessive soil nutrient input, however, reduce the

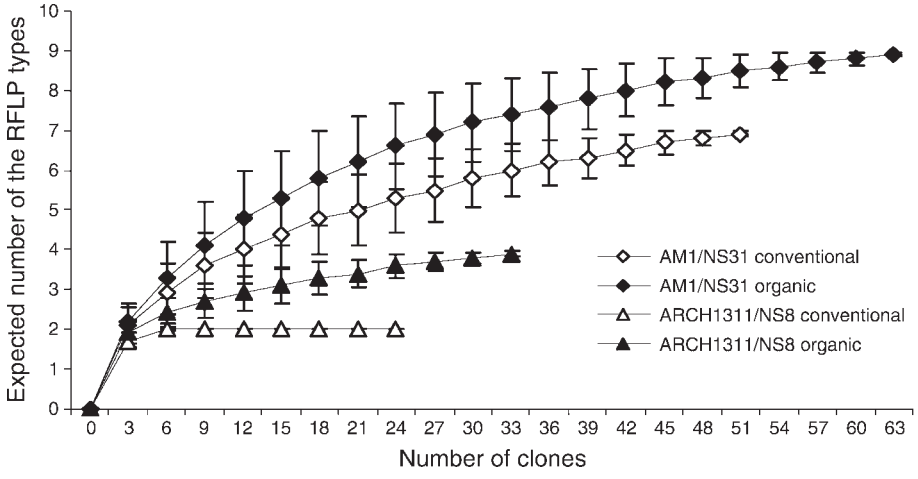

FIG. 2. Rarefaction curves of small subunit (SSU) ribosomal DNA libraries obtained from clover trap culture roots through PCR (polymerase chain reaction) amplification with the NS31/AM1 or ARCH1311/NS8 primer pairs.

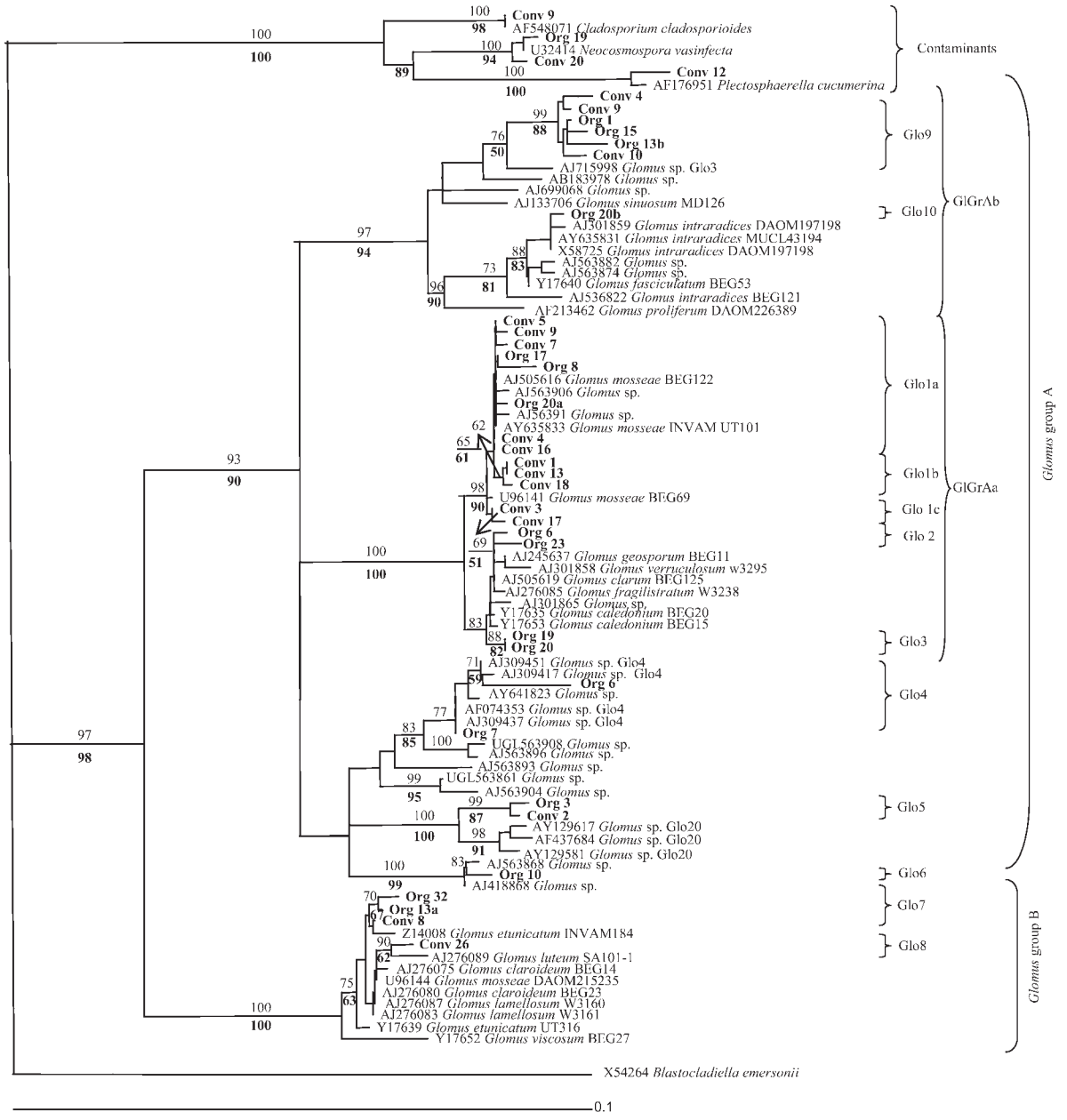

FIG. 3. Neighbor-joining phylogenetic tree showing the relationships among the NS31/AM1 rDNA sequences from the trap culture roots and reference sequences from GenBank. Numbers above the branches indicate the bootstrap values (above 50%, 100 replicates) of the neighbor-joining analysis; numbers below the branches indicate the bootstrap values of the maximum parsimony analysis (in boldface). Individual clones obtained in this study (in boldface) are identified by agricultural system (Conv, Conventional; Org, Organic) and clone number (e.g., 10, 13b). Group identifiers (e.g., Glo1) are the AM fungal sequence types found in our study. *Blastocladiella emersonii*, a zoosporic fungi, was used as the outgroup.

contribution of AM fungi to plant growth and could prevent the use of AM biotechnologies in most productions. Advances in mycorrhizal research have revealed that AM fungi are a heterogeneous group of soil fungi whose positive effects on agricultural production can be enhanced through plant breeding, inoculation, soil fertility and water management. Taking into account the contribution of those AM fungi that are indigenous to agricultural fields and studying the effect of agricultural practices on indigenous AM fungi may offer some insight into the direction modern and sustainable farming should follow.

In the present work we attempted to detect the influence of different soil nutrient conditions and water management practices on indigenous AMF communities in Italian rice fields. In Italy, organic management is

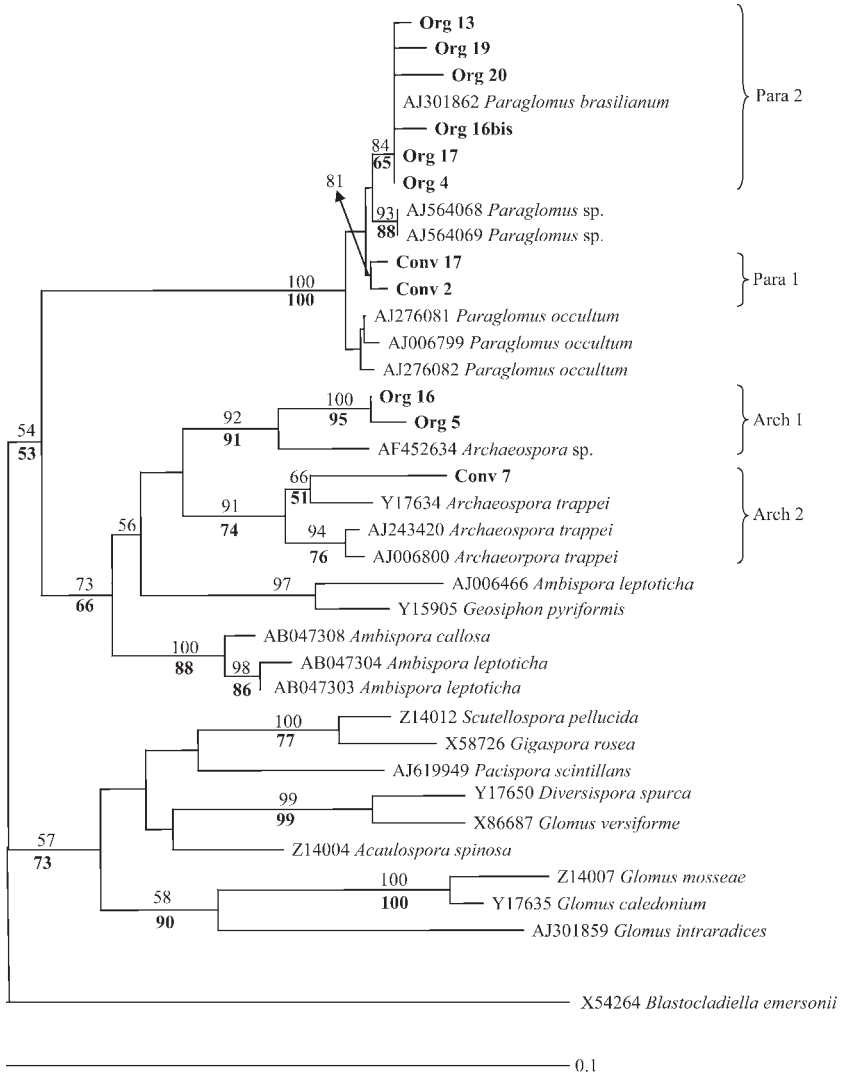

FIG. 4. Neighbor-joining phylogenetic tree showing the relationships among the ARCH1311-NS8 rDNA sequences from the trap culture roots and reference sequences from GenBank. Numbers above the branches indicate the bootstrap values (above 50%, 100 replicates) of the neighbor-joining analysis; numbers below the branches indicate the bootstrap values of the maximum parsimony analysis (in boldface). Individual clones obtained in this study (in boldface) are identified by agricultural system (Conv, Conventional; Org, Organic) and clone number (e.g., 16bis). Group identifiers (e.g., Para2) are the AM fungal sequence types found in our study. *Blastocladiella emersonii*, a zoosporic fungi, was used as the outgroup.

gaining importance, but it is far from being widespread, especially in rice farming, where there is a lack of and a need for specific experimentation. We therefore decided to conduct a pilot study on two adjacent rice farms that adopt two different crop management systems: conventional farming with continuous high-input rice monocropping and organic farming with a five-year crop rotation. This pairing of neighboring organic and conventional fields was performed to ensure that the climatic and soil conditions were approximately the same for this first, small-scale, direct comparison. The farms are located to the West of Pavia, in an area known for the cultivation of rice varieties that are suitable for "risotto." Both the plant size of these varieties and the soil texture make farmers adopt a cultivation technique based on dry seeding and flooding at tillering stage. This technique is suitable for organic practices.

Our data showed that the conventional system depressed the mycorrhizal infection in rice plant roots. In fact, one month after seeding, the rice plants roots did not present fungal colonization under the high-input system; on the contrary, the rice plants grown under the low-input system showed fungal hyphae and arbuscules in the roots, suggesting the presence of active symbiosis. These observations are in agreement with several studies on other crops, which demonstrated that intensive

farming practices are evidently a threat to AMF abundance and effectiveness with respect to root colonization and plant growth promotion which decline upon agricultural intensification. (Helgason et al. 1998, Jansa et al. 2002, 2003, Oehl et al. 2003, 2004, 2005, 2009, Alguacil et al. 2008, 2009).

The rate of AMF rice root colonization increased over the plant life cycle only in the fields subjected to delayed flooding, while the plants grown in the fields flooded at the conventional time (June) showed no root colonization. The absence of rice root AMF colonization in the flooded fields confirms previous observations (Vallino et al. 2009). However, the occurrence of AMF colonization in rice fields under submerged conditions is still not clear. Ilag et al. (1987) stated that it is rare or absent due to the anoxic environment; Barea (1991), instead, concluded that AMF are obligate aerobes in nature, but can survive in waterlogged conditions. Different experiments in pots or nursery conditions showed that AMF rice root colonization is affected by water regimes, using either field soil or soil with added fungal inoculum (Solaiman and Hirata 1995, 1998, Purakaystha and Chhonkar 2001). Cornwell et al. (2001) showed that AM fungi can colonize the plant roots of aquatic plants, but that the mycorrhizal status of monocots and dicots differed greatly: in fact, monocot species are generally not mycorrhizal. They found the higher proportion of aerenchyma in the monocot roots as an explanation of the different patterns in mycorrhizal colonization. The authors proposed, on the basis of two studies on rice cultivars (Kirk and Du 1997, Lu et al. 1999), that aerenchyma, through a high O_2 and H^+ release into soil, may play a role in facilitating P uptake and that this increased P uptake would be responsible for a decrease in colonization. All together these data suggest that, on one end, aerenchyma in rice roots may provide an oxic environment that is suitable for AMF growth and colonization, while, on the other, it may facilitate P uptake which inhibits AMF colonization. Therefore, a delayed flooding and a reduced nutrient input would seem to promote AM symbiosis development in rice fields. It is worth noting that in these experimental trials, organic rice had a very good yield response, thanks to better nutrient availability and higher soil fertility (data not shown).

It is known that waterlogged soils contain sufficient AMF propagules to infect plants when the soil dries (Miller et al. 1999, Miller 2000). We have confirmed these data with the results obtained from trap cultures grown on both soil inocula (organic and conventional systems). The root colonization was very high for both (100%) demonstrating that AMF are still present in the soil and capable of infecting *Trifolium repens* roots.

In order to obtain a picture of AMF biodiversity, we took random fragments of *Trifolium repens* roots and analyzed them molecularly. We are aware that the trap cultures might not have revealed all the AMF diversity because the AMF communities in trap culture roots do not exactly mirror the situation present in field sites (Sýkorová et al. 2007), but some considerations can still be drawn. Since the Shannon-Weaver index resulted to be higher in the organic system ($H' = 1.79$) than the conventional one ($H' = 1.08$), we can conclude that the diversity of the AM fungal community in rice soils is negatively affected by conventional practices, as previously reported for other crop cultures (Oehl et al. 2004). In addition, Verbruggen et al. (2010), in a large-scale comparison of mycorrhizal fungal communities in agricultural soils, have recently remarked on the fact that AMF communities of organically managed sites resulted to be more similar to those of semi-natural grasslands than to those of conventionally managed sites, thus indicating that AMF assemblages have the potential of recovering their natural state after years of intensive agriculture. Taken together, all these data suggest that organic farming appears to be a suitable agricultural management strategy with respect to the beneficial effects on AMF biodiversity.

The NS31-AM1 primer combination used in this study amplified an AMF consortium present in the rice agrosystem composed of various taxa within *Glomus* group A and *Glomus* group B of the *Glomeraceae* family (Schüßler et al. 2001). However, we did not detect the presence of fungal sequences belonging to the other three families (*Acaulosporaceae, Diversisporaceae* or *Gigasporaceae*), which are also targets of this set of primers. The predominance of the *Glomus* genus has been reported in other studies of AMF communities in arable soils (Daniell et al. 2001, Hijri et al. 2006), even in wetland soils (Wirsel 2004). *Glomus* species are capable of colonizing via fragments of mycelium or mycorrhizal root pieces (Daniell et al. 2001); they readily form anastomoses between mycelia and might therefore have the ability of reestablishing an interconnected network after mechanical disruption (Giovanetti et al. 1999). All these facts might explain the dominance of the *Glomeraceae* family in our agricultural conditions, which included chemical applications (such as fertilizers and pesticides) and monoculture.

Some fungal type we recovered could represent new species in this environment, for example Glo9. Others fungal types had already been detected in molecular field studies. This is the case of Glo1 (a,b and c), which was the dominant species in both agroecosystems and corresponded to *G. mosseae*. A predominance of *G. mosseae* has also been reported at various frequencies in different ecosystems (Husband et al. 2002, Wirsel 2004, Hijri et al. 2006, Galván et al. 2009, Rosendahl et al. 2009); it was the major component of AMF communities in many crop plants and it was able to efficiently infect roots in spite of the different agricultural practices used (Helgason et al. 1998). Although *G. mosseae* does not show host preference, it might have a competitive advantage under some conditions (Wirsel 2004). Glo5 matches the Glo20 type, which has been observed in tropical forests (Husband et al. 2002) and temperate

grasslands (Vandenkoornhuyse et al. 2002); the Glo4 type was also recovered from arable crops (Daniell et al. 2001); Glo2 is closely related to a database sequence of *G. geosporum*, whereas Glo10 is closely related to or identical to sequences of *G. intraradices*. These species might exhibit preferences for particular agricultural practices, such as organically managed land. A similar case concerns a sequence recovered in soil belonging to conventional managed land (Glo8) which was identical to sequences of *G. luteum*. Glo6 was also recovered under the organic system only and matched a *Glomus* species discovered by Wirsel (2004) in a temperate wetland ecosystem: these species possess an ability to survive in wetland conditions that may be unfavorable for other AM fungi.

In order to detect and identify all the currently known AM fungi in the *Glomeromycota* phylum, we also included an assay that specifically targeted members of the *Paraglomus* and *Archaeospora* genera in our study, using the ARCH1311-NS8 primer combination. The most abundant fungal type found in the inoculum from the organic system could be assigned to *Paraglomus brasilianum* and one sequence found in the inocula of the conventional system was assigned to *Archaeospora trappei*. This last species was also detected by Hijri et al. (2006) in a study on AMF communities in arable soils. They only found this species in trap cultures but not in field collected roots, which is in agreement with the results of other molecular studies on AM communities in Central Europe (Daniell et al. 2001, Vandenkoornhuyse et al. 2002, Scheublin et al. 2004, Renker et al. 2005, Börstler et al. 2006). This finding indicates that the conditions in pot cultures could favor certain AMF taxa (Sýkorová et al. 2007), even though some recent works have reported the presence of different *Archaeospora* phylotypes associated to diverse environments, such as salt marshes (Wilde et al. 2009), serpentine soils (Schechter and Bruns 2008) and organic farm soils (Galvan et al. 2009). The others sequences obtained with the primers sets could not be assigned to known species and could represent new fungal types belonging to *Archaeosporales* or Paraglomerales, whose phylogenetic placement remain ambiguous (Opik et al. 2010).

In conclusion, we have provided a snapshot on the biodiversity of AMF in a rice rhizosphere in relation to two different agricultral practices: conventional farming with continuous high-input rice monocropping and organic farming with a 5-year crop rotation. We have shown that AMF communities of *Glomerales*, *Paraglomerales* and *Archaeosporales* are present in the soil of both types of agricultural systems and that their assemblage composition is influenced by management practices. Our results suggest that high input cropping systems and conventional flooding depress AM fungal colonization in rice roots and that organic managements may help maintain a higher diversity of AM fungal communities in soil.

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APPENDIX

Images showing sampling sites and design (*Ecological Archives* A021-077-A1).