



Agricultural abandonment in Mediterranean reclaimed peaty soils: long-term effects on soil chemical properties, arbuscular mycorrhizas and CO₂ flux



Elisa Pellegrino ^{a,1,*}, Simona Bosco ^{a,1}, Valentina Ciccolini ^{a,1}, Chiara Pistocchi ^{a,b}, Tiziana Sabbatini ^a, Nicola Silvestri ^c, Enrico Bonari ^a

^a Institute of Life Sciences, Scuola Superiore Sant'Anna, P.za Martiri della Libertà 33, 56127 Pisa, Italy

^b Group of Plant Nutrition, USYS ETH Zurich, Eschikon 33 Lindau, Switzerland

^c Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

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ABSTRACT

In the last century, most peatlands were reclaimed for agricultural purposes, which led to peat degradation and to severe subsidence, and thus too wet conditions for crops. In some areas this has therefore led to wide agricultural abandonment. However, studies on the effect of agricultural abandonment as a potential restoration tool are lacking. In this study, the effectiveness and the restoration potential of agricultural abandonment in reducing peat degradation and in improving soil microbial biodiversity were evaluated. The main chemical parameters, arbuscular mycorrhizal (AM) fungal diversity and soil respiration partitioning were used to assess the long-term effect of 15 years of agricultural abandonment (Aband) in a Mediterranean reclaimed peatland. An intensive maize cultivation (Cult) in the same area was used as a comparison. Multivariate analyses showed that 15 years of agricultural abandonment: did not affect the main soil chemical parameters, except for NH₄⁺ which was lower in the Aband than in the Cult; increased AM fungal root colonization and the diversity in terms of number of families of AM fungi retrieved in roots, but decreased soil AM fungal richness; reduced total soil respiration and its autotrophic component but increased respiration by heterotrophs; determined a lower fluctuation of soil CO₂ flux response to air temperature than the Cult. Thus, although some soil quality parameters were significantly improved, 15 years of agricultural abandonment may not lead to an effective restoration. Consequently, alternative and sustainable solutions for the protection and preservation of Mediterranean peatlands need to be developed.

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1. Introduction

Wetlands are species-rich habitats performing valuable ecosystem services such as flood protection, water quality enhancement and carbon (C) sequestration. Their protection is officially a priority for 168 nations that have ratified the Ramsar Convention (Verhoeven and Setter, 2010). These habitats cover about 6% of the global surface area and about 60% are represented by peatlands, which play an important role in the global C cycle as a long-term sink. In Europe peatlands cover about 20% of the land area. Although most are located in northern Europe, some small sites are also situated in the Mediterranean area (Montanarella et al., 2006).

In the last 200 years, most peatlands have been reclaimed in Europe for agricultural purposes because of their natural fertility, which has thus destroyed their original character and ecological functions (Verhoeven and Setter, 2010). The increase in aerobic conditions due to the reclamation and agricultural practices led to significant increases in soil organic matter (SOM) oxidation. Major consequences included the high release of carbon dioxide (CO₂), nutrient losses to water bodies, biodiversity losses and degradation of the peat with a drastic decrease in soil quality together with a severe subsidence which led in some areas to conditions that are too wet for crops.

Soil quality can be evaluated using chemical, biochemical and biological indicators (Doran and Parkin, 1996). Within the chemical and biochemical parameters of soil, SOM and soil respiration are the main and most suitable indicators to measure C storage and degradation. As regards soil respiration, CO₂ flux is considered as a proxy for SOM decomposition and its partitioning between the

* Corresponding author. Tel.: +39 050 883181; fax: +39 050 883526.

E-mail address: e.pellegrino@sssup.it (E. Pellegrino).

¹ These authors contributed equally to this work.

microbial and the root component is required in order to identify CO₂ sources and to calculate their contribution to the total flux (Kuzayakov, 2006). In addition, in order to assess soil health and functioning, an evaluation of the diversity of beneficial microbes such as arbuscular mycorrhizal (AM) fungi is valuable since these fungi are involved in plant growth and nutrient uptake enhancement and in improving soil structure (Smith and Read, 2008).

Several studies have investigated the soil quality degradation of reclaimed peatlands intensively used for agriculture. Numerous strategies have been proposed for their restoration, such as the rewetting with or without the introduction of plant species, and a shift to extensive-grazing systems or to less intensive agriculture use (Pfadenhauer and Grootjans, 1999; Zeitz and Velty, 2002). In the agricultural peatlands where none of these strategies have been adopted, the subsidence and consequent inability to support crop growth have led to the massive abandonment of the areas (Joosten and Clarke, 2002).

However, studies are lacking on the effect of the agricultural abandonment in reclaimed peatlands, and in particular on the most vulnerable Mediterranean areas where climatic conditions favor a rapid mineralization of the SOM. Therefore, the effectiveness and the restoration potential of the agricultural abandonment in reducing the peat degradation and in improving soil microbial biodiversity need to be evaluated.

We assessed the long-term effect of 15 years of agricultural abandonment on the soil quality of a Mediterranean reclaimed peatland located in the Massaciuccoli Lake basin (Tuscany, Italy). To this end, chemical parameters, AM fungal molecular diversity and community structure, and *in situ* soil respiration partitioning were used. The agricultural abandonment was compared with an intensively cultivated peaty soil represented by a continuous maize (*Zea mays* L.) cropping system, which is the main system in the area (Silvestri et al., 2012).

2. Materials and methods

2.1. Field site

The experimental site is situated in the south of the Massaciuccoli Lake basin (43°49'N–10°19'E) within the natural park of Migliarino-San Rossore-Massaciuccoli (Pisa, Italy). Since 1930, most of the basin has been drained by a complex network of artificial canals, ditches and pumping stations. This has forced water from reclaimed areas into the lake, causing a severe subsidence ranging from 3 to 4 cm year⁻¹, caused by compaction and peat oxidation (Pistocchi et al., 2012). The soil was classified as *Histosol* according to the USDA system (Soil Survey Staff, 1975) and as *Rheic Histosol* according to the FAO system (IUSS, 2006). The thickness of the organic horizon is about 3 m (from 2 to 4 m) with a SOM content of 29.2% (minimum 20.1%–maximum 55.4% (Walkley-Black)), a clay content of 25%, a pH of 4.9, a bulk density of 0.5 g cm⁻³ in the 0–30 cm depth layer. Therefore, these organic soils can be defined also as peat and peaty soil (IPCC, 2006).

During the year the water table is maintained by pumping stations at a quite stable level, ranging from 0.40 to 0.60 m (Pistocchi et al., 2012). Therefore, the upper soil layer is only occasionally subjected to water saturation. The climate is Mediterranean (Csa) according to the Köppen–Geiger climate classification map (Kottek et al., 2006). Summers are dry and hot, rainfall is mainly concentrated in autumn and spring (mean annual rainfall ca. 945 mm year⁻¹) and mean monthly air temperature at 2 m ranging from 7 °C in February to 30 °C in August (with a mean of 14.8 °C year⁻¹). Average monthly maximum, mean and minimum temperatures and rainfalls over the period 1990–2012, recorded at a weather station located in the Massaciuccoli basin are shown in Fig. S1. The site was cultivated with maize until

15 years ago when agricultural practices were abandoned in an area of about 15 ha and the natural succession vegetation were left to develop. The surrounding area (about 15 ha) is still cultivated with maize.

2.2. Experiment 1: effect of agricultural abandonment on chemical parameters, root traits and AM fungi

The aim of this experiment was to evaluate the long-term effect of the abandonment of agricultural practices compared to maize cultivation in terms of chemical soil parameters, root traits, such as the root biomass and AM fungal colonization, and AM fungal diversity and community structure.

2.2.1. Experimental set-up

The experiment was a completely randomized design with land use as treatment and three replicates ($n=3$; field replicates of 0.7 ha) (Fig. S2). We had six cases (field replicates), three of them of one land use and another three of the other land use. At the beginning, before the experiment started, fields looked all the same and we had numbered them 1–6 and let a random number generator to pick the three fields that received same treatment. The first land use type was an agricultural peaty soil left abandoned for 15 years (Aband). Field replicates were left to develop under the natural succession vegetation. A floristic survey showed that the most common species were *Abutilon theophrasti* L., *Amaranthus retroflexus* L., *Arctium lappa* L., *Artemisia* sp., *Atriplex* sp., *Bidens* sp., *Biphora* sp., *Calystegia* sp., *Datura stramonium* L., *Echinochloa crus-galli* L., *Galium* sp., *Humulus lupulus* L., *Linaria* sp., *Phragmites australis* L., *Typha latifolia* L., *Lythrum salicaria* L., *Phytolacca americana* L., *Rumex crispus* L., *Silene alba* L. and *Xanthium* sp. The most abundant plant species were *Calystegia* sp. (17.5%), *P. australis* (15%), *A. lappa* (13.8%) and *B. tectorum* (13.8%). No fertilizers or other agricultural practices were applied, except for an annual vegetation cutting at the end of the winter season.

The second land use type was an intensively cultivated peaty soil represented by continuous maize (Cult). Each year in late spring field replicates were ploughed (30–35 cm) and harrowed, as the main and secondary tillage, respectively. Maize was sown at the beginning of June at a rate of 75,000 seeds ha⁻¹ with 75 × 17 cm row spacing and harvested in late September. Fertilization was applied at sowing and at mechanical weeding with rates of 32 kg ha⁻¹ N, 96 kg ha⁻¹ P, 96 kg ha⁻¹ K and 138 kg ha⁻¹ N, respectively. Chemical and mechanical post-emergence weed controls were applied. The fifteen year average maize yield was 6.4 t ha⁻¹.

2.2.2. Sampling

In July 2011 one combined soil sample, resulting from pooling seven soil cores, was collected from each field replicate (0–30 cm depth) in order to cover chemical and AM fungal spatial variability. Sampling was carried out only once in July, since mid-summer is the best choice because sampling should not be close to soil treatments and because the variability in chemical parameters changes slightly during the year (Pellegrino et al., 2011). These facts, along with the fact that AM fungi consistently maintain the same patterns of variability in differently managed systems, although with seasonal changes, were taken into account when choosing July as a single sampling time (Vandenkoornhuysen et al., 2002; Oehl et al., 2010; Di Bene et al., 2013).

Soil samples used for the chemical parameter determinations were oven dried at 60 °C and then sieved at 2 mm, while for genomic DNA extraction roots were carefully plucked with forceps. Soil DNA extracts were stored at 4 °C. As regards the root trait determinations, seven turfs were extracted (20 cm depth) from each field replicate and then combined. In the laboratory, roots were collected from each combined sample, then washed and

dried at 60 °C for root dry weight (DW) measurements, whereas for AM fungal root colonization assessment and genomic DNA extraction, root subsamples were taken and stored at 4 °C.

2.2.3. Soil chemical analyses

Soil samples were analyzed for: pH; electrical conductivity, EC; exchangeable potassium, K_{exch} ; total nitrogen, N_{tot} ; ammonium, NH_4^+ ; nitrates, NO_3^- ; soil organic matter, SOM; total phosphorus, P_{tot} ; available phosphorus, P_{avail} and organic phosphorus, P_{org} . Soil pH and EC were measured in deionized water (1:2.5 and 1:2, w/v, respectively). K_{exch} was determined by atomic absorption. P_{tot} and P_{avail} were determined by colorimetry using perchloric acid digestion and an extraction with sodium bicarbonate, respectively (Olsen and Sommers, 1982). P_{org} was evaluated using the Metha extraction (Hence and Anderson, 1962). N_{tot} was determined by the macro Kjeldahl digestion procedure, while NO_3^- and NH_4^+ were determined by the Kjeldahl method after KCl 2 N extraction and, in the case of NO_3^- , after reduction with Devarda's alloy. SOM was measured using the modified Walkley-Black wet combustion method (Nelson and Sommers, 1982). Soil C/N ratio was calculated by dividing SOC ((SOM/1.7) × 10) by total N.

2.2.4. Root determination and AM fungal root colonization

From the combined turfs of each field replicate, soil subsamples (mean soil DW ca. 400 g) were used to determine root DW. Roots were manually collected with forceps and washed by wet-sieving and decanting down to a mesh size of 250 μm. After removing organic debris, all live and dead root fragments were oven-dried and weighed to determine root DW. Root DW per gram of soil was calculated.

AM fungal root colonization was assessed under a stereomicroscope (Olympus SZX 9, Olympus Optics, Tokyo, Japan), after clearing and staining with lactic acid instead of phenol (Phillips and Hayman, 1970), following the gridline intersect method (McGonigle et al., 1990). The roots were mounted on microscope slides and examined at magnifications of 125–500, and verified at a magnification of 1250.

2.2.5. AM fungal diversity: extraction of genomic DNA, PCR amplification, cloning and sequencing

Soil DNA was extracted from 0.5 g of soil using the PowerSoil[®] MoBio kit (Mo Bio Laboratories Inc., NY, USA) ($n=6$), while root DNA was extracted from 100-mg fresh root samples using the DNeasy[®] Plant Mini Kit ($n=6$) (Qiagen, Germantown, MD, USA). DNA quality was checked on a ND-1000 spectrophotometer (NanoDrop Technology, Wilmington, DE). The direct extraction of the DNA from soil was performed to prevent the host preference effect as previously described by Davison et al. (2012). PCR amplification was performed using the primer pair NS31 and AM1 targeting the small subunit ribosomal RNA (SSU rRNA) region (Simon et al., 1992; Helgason et al., 1998). Although longer and higher discriminating regions are available (Krüger et al., 2012; Pellegrino et al., 2012), the NS31/AM1 SSU region was targeted because most Glomeromycota diversity data are obtained using this region, which provides a larger comparative DNA sequence data-set.

PCR was performed using the temperature profile described by Helgason et al. (1998). No PCR inhibition problems were registered. PCR amplicons were generated from 10 ng μL⁻¹ genomic DNA in volumes of 20 μL with 0.5 U of GoTaq[®] hot start polymerase (Promega Corporation, Madison, WA, USA), 0.2 μM of each primer (NS31/AM1), 0.2 mM of each dNTP, 1.25 mM of MgCl₂ and 1x reaction buffer, using the S1000 Thermal Cycler[™] (BIORAD, USA).

Before ligation, the quantity and quality of the PCR amplicons were checked by a spectrophotometer (NanoDrop Technology, Wilmington, DE). The PCR amplicons were then ligated into the

pGem[®]-T Easy vector (Promega Corporation, Madison, WA, USA) and used to transform XL10-Gold[®] Ultracompetent *Escherichia coli* cells (Stratagene[®], La Jolla, CA, USA). At least 25 recombinant clones per amplicon library ($n=12$) were screened for the c. 550-bp-long NS31/AM1 fragment on agarose gels.

A total of 270 PCR products obtained from clones (a mean of 23 per library) were sequenced using the NS31/AM1 primers in an ABI Prism[®] 3730XL automated sequencer (Applied Biosystem, Foster City, CA, USA) at the High-Throughput Genomics Unit (Seattle, WA, USA).

2.3. Experiment 2: effect of agricultural abandonment on soil CO₂ flux

The aim of this experiment was to evaluate the long-term effect of agricultural abandonment compared with maize cultivation on total soil respiration flux and autotrophic and heterotrophic CO₂ flux components.

2.3.1. Experimental set-up and sampling

The experimental design was as described above for experiment 1. Six experimental blocks (one for each field replicate) were established for soil respiration monitoring (Fig. S3 a,b). Six blocks were considered as an adequate number due to the texture homogeneity of the mineral component of the soil (Table S1). Blocks were located about 300 m apart. In each field plot, the block consisted of two 20-cm diameter open-ended PVC collars: a surface collar (7.0-cm deep collar inserted 1 cm into the soil) pressed firmly onto the shallow surface layer without cutting any roots (Fig. S3a); a 25-cm deep collar inserted 20 cm into the soil (Fig. S3b). The collar depth was evaluated as being appropriate to exclude 90% of fine roots from the soil volume (Heinemeyer et al., 2007). Plants inside the collars were removed, leaving the root systems intact. The surface and the deep collars provided a measure of the total soil respiration (Rs) and of the respiration by heterotrophs (Rh, soil microorganisms and mesofauna), respectively (Heinemeyer et al., 2007, 2011). These measurements were used to calculate the contribution of the root component defined as respiration by autotrophs ($R_a = R_s - R_h$).

Soil CO₂ flux was measured using a non-steady-state through-flow chamber equipped with a portable infrared gas analyzer (IRGA) (Licor LI-820) connected to a steel chamber with a headspace volume of 6186 cm³ (chamber B, West Systems Srl, Pontedera, Italy). To guarantee a tight seal with the collars, the chamber had a rubber ring that fits into the collar lip. The CO₂ concentration was measured per second, while the increase in the headspace (ppm/s) was checked for linearity for a period of

Table 1

Soil chemical parameters (0–30 cm depth) of abandoned agricultural peaty soils (Aband) and of a maize cultivation (Cult).

Chemical parameters	Aband	Cult
pH [*]	5.3 ± 0.4 ^{**}	4.6 ± 0.1
EC (mS cm ⁻¹)	0.9 ± 0.1	1.8 ± 0.9
K_{exch} (mg kg ⁻¹)	560.0 ± 84.6	397.0 ± 51.9
N_{tot} (g kg ⁻¹)	11.8 ± 1.2	13.0 ± 2.2
NO_3^- (mg kg ⁻¹)	59.0 ± 18.6	42.3 ± 15.7
NH_4^+ (mg kg ⁻¹)	56.3 ± 4.5 a	148.0 ± 9.6 b
SOM (%)	28.3 ± 4.6	25.7 ± 4.1
C/N	13.7 ± 1.0	11.5 ± 0.3
P_{tot} (mg kg ⁻¹)	2846.7 ± 229.2	2709.0 ± 329.3
P_{avail} (mg kg ⁻¹)	76.3 ± 13.6	70.3 ± 7.9
P_{org} (mg kg ⁻¹)	2054.7 ± 198.1	2153.3 ± 363.4

^{*} EC: electrical conductivity; K_{exch} : exchangeable potassium; N_{tot} : total nitrogen; NO_3^- : nitrate; NH_4^+ : ammonium; SOM: soil organic matter; C/N: carbon/nitrogen ratio; P_{tot} : total phosphorus; P_{avail} : available phosphorus; P_{org} : organic phosphorus.

^{**} Values are means ± SE of three field replicates for each treatment. Values in the same row followed by different letters are statistically different between land uses according to ANCOVA ($P < 0.05$).

2–3 min, and calculated and recorded in the field by a palm top computer connected via Bluetooth. CO₂ flux was calculated by performing linear regressions on the logged CO₂ data ($R^2 > 0.95$), which were corrected for atmospheric pressure and air temperature. An internal fan maintained the homogeneity of the air mixture within the chamber during the measurements. Soil moisture and temperature were recorded at each measurement next to each collar by a probe (Decagon Devises ECH₂O-TE/EC-TM) inserted into a soil depth of 5 cm.

In 2012 monitoring was undertaken between 14 May and 13 August with a measurement frequency of once or twice per week (a total of 21 measurements per block). The sampling period was chosen by taking into consideration that in order to evaluate the impact of a land use change on soil biochemical parameters, it is necessary to sample at a much later date from soil treatments (Picci and Nannipieri, 2002). Daily mean temperatures and rainfalls during the sampling period (from May to August 2012) were recorded (Fig. 3a). Measurements were taken between 8 a.m. and 12 a.m., because mid-day values of CO₂ flux are assumed to be representative of a daily average flux (Davidson et al., 1998; Luo and Zhou, 2006). Sampling started ten days after collar insertion, which is a sufficient time lapse to allow for considerable root death and to exclude the Ra component from deep collar.

2.3.2. Modeling the soil respiration

The cumulated Rs and Rh (from 14 May to 13 August 2012) were calculated using the exponential relationship between soil CO₂ flux and air temperature. The Van't Hoff empirical exponential equation (Q10), a simplified version of the Rothamsted Carbon Model (RothC), with temperature as an independent variable (Coleman and Jenkinson, 1999) and the Lloyd and Taylor (LT) (Lloyd and Taylor, 1994) models were used in order to assess the sensitivity of soil respiration to air temperature. The three models were fitted on the measured soil CO₂ flux of Rs and Rh for both land uses, and were then correlated with air temperature.

The performance of the three models of CO₂ flux response to air temperature was evaluated using the Akaike information criterion (AIC); the root mean squared error (RMSE) and the adjusted R-square value (Rsd. ad). The best statistical fit was chosen to calculate the cumulative flux.

2.4. Data and sequence analysis

An updated AM fungal reference dataset of 59 NS31/AM1 public sequences (ca. 550 bp) was created using only morphotype sequences, including the majority of the AM fungal species listed in Schüßler and Walker (2010) phylotaxonomic classification (this alignment is in an open-access database <https://sites.google.com/site/restomedpeatland/microbiology>). The reference dataset and their correspondence with the closest (similarity higher than 99%) virtual taxon (VT) after blast search against the MaarjAM database (Öpik et al., 2010) is shown in Fig. S4 and Table S2. The AM fungal reference dataset was used for the further alignment of the newly-generated sequences using Bioedit (Hall, 1999), after having checked the quality of their electropherograms by Vector NTI

Advance 10 (Invitrogen, USA) and affiliation to the Glomeromycota using a basic local alignment search tool (BLAST) search (Altschul et al., 1997). An alignment of a total of 195 sequences (21 from the reference dataset, 13 from NCBI after blast search (similarity higher than 99%), 160 newly generated sequences and the *Corallochytrium limacisporum* sequence L42528 as the outgroup) was trimmed to the same length (ca. 490 bp) and manually refined. Phylogenetic trees were inferred by the neighbor-joining (NJ) analysis using MEGA version 5.1 (Tamura et al., 2011 <http://www.megasoftware.net>) and the Kimura 2-parameter model (Kimura, 1980). Branch support values correspond to 1000 bootstrap replicates. The phylograms were drawn by MEGA 5.1 and edited by Adobe Illustrator CS6.

The phylogram was used to assign the newly-generated AM fungal sequences to molecular operational taxonomic units (MOTUs) on the basis of a bootstrap value of 75. In addition, the correspondence of each MOTU with the closest VT (Öpik et al., 2010) was computed. AM fungal MOTU richness and the Shannon index (H') were calculated using Primer v6 (Clarke and Gorley, 2006 <http://www.primer-e.com>). The suitability of the AM fungal community sampling was verified by Coleman rarefaction curves (Coleman, 1981) in EstimateS version 9.1 (Colwell, 2013 <http://purl.oclc.org/estimates>) using individual-based rarefaction curves (Gotelli and Colwell, 2001) with clones/sequences considered as units of replication.

All newly-generated sequences were submitted to the EMBL nucleotide sequence database (<http://www.ebi.ac.uk/embl/>) and are available under accession numbers HG425705–HG425864.

To compensate the scarce interspersions of the field replicates generated by our randomization and to prove that the effects that we detected were unlikely to have been caused by a purely spatial phenomenon, we accounted for plot positions by applying the analysis of covariance (ANCOVA). Land use was used as fixed factor and the spatial coordinates of the plots (latitude/longitude) as covariables. Therefore, we factored out the distance variable that may affect the studied dependent variables, allowing a proper comparison of the treatments. Spatial coordinates were first transformed into radians and then standardized. All the dependent variables were log- or arcsin-transformed when necessary to fulfill the assumption for the ANCOVA. When the assumptions for the ANCOVA were not fulfilled, even after the appropriate transformation, data were analyzed using the Mann–Whitney non-parametric test or the Kruskal–Wallis H non-parametric test, followed by the Mann–Whitney test. All these analyses were performed in SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Constrained ordination analyses (partial redundancy analysis, pRDA) were used to investigate the influence of the different land uses (used as explanatory variables) on the chemical, root and CO₂ parameters and on the AM fungal relative abundances of MOTUs (used as response variables) accounting for the spatial coordinates of the plots (used as covariables). In partial constrained analysis, information that can be explained by the covariables is first extracted and then the explanatory variables are used to “explain” the residual variation. RDAs were used to assess the influence of different matrices (roots and soil) on the explanatory variables. RDA linear method (Lepš and Šmilauer, 2003) was used since the gradient of the detrended correspondence analysis was lower than four. All data were log-transformed, centered and standardized by the response variables. Monte Carlo permutation tests were performed using 499 random permutations (unrestricted permutation) in order to determine the statistical significance of the relations between land use and response variables. RDAs and pRDAs were performed by Canoco for Windows v. 4.5 (ter Braak and Šmilauer, 2002). The biplots were drawn by CanoDraw for Windows.

Table 2

Root dry weight and arbuscular mycorrhizal (AM) fungal root colonization of the natural succession vegetation and of the maize occurring in abandoned agricultural peaty soils (Aband) and in a maize cultivation (Cult), respectively.

Parameters	Aband	Cult
Root dry weight (mg g ⁻¹ soil)	1.1 ± 0.5 ^a	0.6 ± 0.2
AM fungal root colonization (%)	33.2 ± 2.0 b	22.2 ± 1.7 a

^a Values are means ± SE of three field replicates for each treatment. Values in the same row followed by different letters are statistically different between land uses according to ANCOVA ($P < 0.05$).

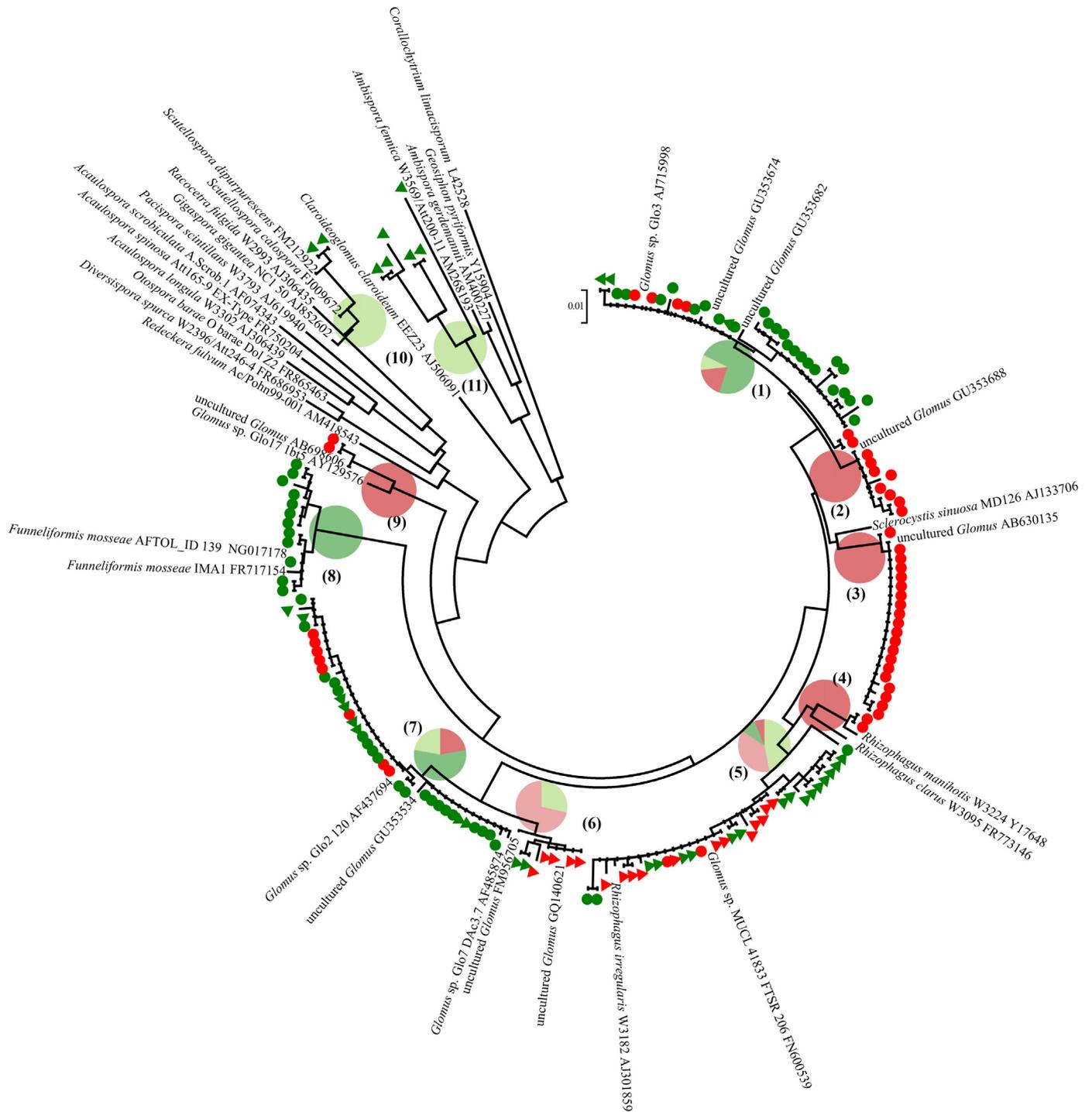


Fig. 1. Neighbor-Joining (NJ) tree of arbuscular mycorrhizal fungal (AMF) sequences derived from roots and soil (shown as triangle and circle, respectively) of agricultural abandoned peaty soils (Aband) and of a maize cultivation (Cult) (green/open and red/filled symbols, respectively). The analysis is based on partial nuclear small subunit ribosomal RNA gene sequences (SSU \approx 550 bp; NS31/AM1 fragment), and the tree is rooted with a reference sequence of *Corallochytrium limacisporum* (L42528). AMF sequences were classified in 11 molecular taxonomic units (MOTUs) affiliated to: *Funneliformis* sp. (8), *Rhizophagus* spp. (4, 5), *Sclerocystis* sp. (3), *Scutellospora* sp. (10) and to additional taxa, such as *Glomus* spp. (1, 2, 6, 7, 9) or an uncultured Glomeromycota (11). Pies indicate the proportions of sequences into the two land uses (Aband, green; Cult, red) and matrixes (roots, light color; soil, dark color). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Bootstrapping is based on 1000 replicates. The analysis involved 195 nucleotide sequences. Evolutionary analyses were conducted in MEGA5. Sequences obtained in the present study are shown by symbols and their accession numbers are shown in Fig. S3. The correspondence between MOTUs and the closest virtual taxa (VT) after blast search against the MaarJAM database (Öpik et al., 2010) is shown in Fig. S3 and Table 3.

Table 3

List of the molecular taxonomic unit (MOTU) of the sequences retrieved in this study and their correspondence with the closest virtual taxon (VT) after blast search against the MaarjAM database (Öpik et al., 2010). The accession number of MaarjAM type sequences is also shown.

MOTU	Code ^a	VT	Accession number
Fun1_AMASS	8	VT065/067	Y17635
Glo1_AMASS	1	VT309	FJ194511
Glo2_AMASS	2	VT309	FJ194511
Glo3_AMASS	6	VT093	EU332715
Glo4_AMASS	7	VT219	AM849279
Glo5_AMASS	9	VT419	EU340305
Rhizo1_AMASS	4	VT090	Y17648
Rhizo2_AMASS	5	VT113/114	AJ418876
Sclero1_AMASS	3	VT310	FJ194510
Scut1_AMASS	10	VT049	AF074340
Uncult1_AMASS	11	VT242/151	AB015052

^a Code number used in Fig. 1.

3. Results

3.1. Experiment 1: effect of agricultural abandonment on soil chemical parameters, roots and AM fungi

3.1.1. Soil chemical parameters, root dry weight and AM fungal root colonization

Fifteen years of agricultural abandonment (Aband) did not significantly modify soil quality, as measured by its main chemical parameters, compared with maize cultivation (Cult), except for soil NH_4^+ concentration which was around three-fold lower in the Aband than in the Cult (Table 1). However, it is noteworthy to highlight an increasing trend in the Aband soil organic matter content, which increased by 9% in comparison with the Cult, as well as other chemical parameters, such as the P_{tot} and P_{avail} , which increased by 8% and 5%, respectively.

As regards roots, DW values were not significantly different between land uses, whereas AM fungal root colonization was significantly higher (30%) in the Aband than in the Cult (Table 2).

3.1.2. AM fungal diversity

The PCR primer pair NS31/AM1, which targets the 3' end of the SSU rRNA gene (\approx 550 bp), was used to amplify and screen the clone libraries obtained from the 12 crude DNA extracts of field root and soil samples. A total of 366 clones were screened and 270 showed the expected AM fungal band length. 132 positive clones were obtained from the Aband (63 and 69 from root (R) and soil samples (S), respectively) and 138 positive clones were obtained from the Cult (65 and 73 from R and S, respectively). After BLAST checking, about 40% of the sequences were excluded due to sequencing errors or PCR primer unspecificity.

The obtained sequences were grouped into 11 different AM fungal molecular operational taxonomic units (MOTUs) (a total of seven and eight in the Aband and the Cult, and six and eight in roots and soil, respectively), which were phylogenetically affiliated

Table 4

Arbuscular mycorrhizal (AM) fungal molecular operational taxonomic unit (MOTU) richness and Shannon (H') index within the native plant species roots and the soil of abandoned agricultural peaty soils (Aband) and within the maize roots and the soil of a maize cultivation (Cult).

Parameter	Roots		Soil	
	Aband	Cult	Aband	Cult
MOTU richness	4.3 \pm 1.7 ^a	1.7 \pm 0.3 A	3.3 \pm 0.3 a	6.0 \pm 0.6 b B
Shannon index (H')	1.1 \pm 0.5	0.4 \pm 0.2 A	1.0 \pm 0.1	1.5 \pm 0.2 B

^a Values are means \pm SE of three field replicates for each treatment. Values in the same row followed by different small letters are statistically different between land uses, according to ANCOVA ($P < 0.05$).

to *Funneliformis* sp. (Fun1_AMASS), five different *Glomus* spp. (from Glo1_AMASS to Glo5_AMASS), two *Rhizophagus* sp. (Rhizo1_AMASS and Rhizo2_AMASS), *Sclerocystis* sp. (Sclero1_AMASS), *Scutellospora* sp. (Scut1_AMASS) and an uncultured Glomeromycota (Uncult1_AMASS) (Fig. 1; Fig. S5). The correspondence between our MOTUs and the closest (similarity higher than 99%) VT after blast search against the MaarjAM database is shown in Table 3. Three out of eleven MOTUs, although three were doubletons (Glo5_AMASS, Rhizo1_AMASS and Scut1_AMASS), were considered for further analyses.

The rarefaction curves showing the relation between the number of sequences and the number of observed AM fungal MOTUs retrieved from roots and soil of the Aband and the Cult (Fig. S6) demonstrated that the sampling effort was sufficient as the accumulation curves reached the asymptote.

As shown in the pie charts in Fig. 1, three AM fungal MOTUs were exclusively retrieved in the Aband: Fun1_AMASS in the soil and the others (Scut1_AMASS and Uncult1_AMASS) within the roots. Four AM fungal MOTUs were only found in the soil of the Cult (Glo2_AMASS, Glo5_AMASS, Rhizo1_AMASS and Sclero1_AMASS). By contrast, Rhizo2_AMASS, Glo1_AMASS, Glo3_AMASS and Glo4_AMASS showed a ubiquitous behavior.

The MOTU richness and Shannon biodiversity index (H') were calculated to evaluate the AM fungal community richness and diversity in the two different land-uses, considering in this way, not only the number, but also the relative proportions of taxa (Table 4). We observed a significantly lower AM fungal richness in the soil of the Aband than in the Cult. Interestingly, in the maize cultivation, the soil showed higher values of both indexes in terms of the roots. Although a differential trend was found and both diversity indexes were three-fold higher in the Aband than in the Cult, 15 years of agricultural abandonment did not significantly affect AM fungal community richness and evenness in the roots (Table 4).

pRDAs showed that land use change significantly affected only the soil AM fungal composition and structure, and that the different matrixes have different AM fungal assemblages. pRDA revealed that land use explained 87.2% (I and II axes) of the whole variance (Fig. 2a), and that its effect on the AM fungal community was significant ($P = 0.036$). As highlighted by the arrows, Glo1_AMASS and Fun1_AMASS showed a preferential presence in the Aband, Glo2_AMASS and Sclero1_AMASS in the Cult (Fig. 2a).

As regards the Cult and the Aband, RDAs showed that the different matrixes explained 89.7% and 89.2% (I and II axes) of the whole variance, respectively (Fig. 2b,c), and that their effect on the AM fungal community was significant ($P = 0.002$). As shown by the arrows, in the Cult Glo2_AMASS, Sclero1_AMASS, Glo1_AMASS, Glo4_AMASS, Glo5_AMASS and Rhizo1_AMASS are soil preferential, while Glo3_AMASS and Rhizo2_AMASS are root preferential (Fig. 2b). Also in the Aband, the arrows showed that Rhizo2_AMASS and Glo3_AMASS are preferential of the roots and that Glo1_AMASS of the soil (Fig. 2c). Interestingly, this RDA biplot highlighted that Scut1_AMASS/Uncult1_AMASS and Fun1_AMASS, exclusively present in the Aband, were preferential of the roots and of the soil, respectively.

3.2. Experiment 2: effect of agricultural abandonment on soil CO_2 flux

3.2.1. Soil CO_2 flux measurements

Both air and soil temperatures and soil moisture varied considerably during the monitoring period (Fig. 3a,b). From May to August, the mean air and soil temperatures ranged from 12.0 °C to 26.6 °C (average value over the period 21.5 °C) and from 16.1 °C to 30.2 °C, respectively; soil moisture (% v:v) decreased in both land uses from 27.5% to 2.2% and from 30.8% to 15.8% for the Aband and the Cult, respectively (Fig. 3b). Rs,

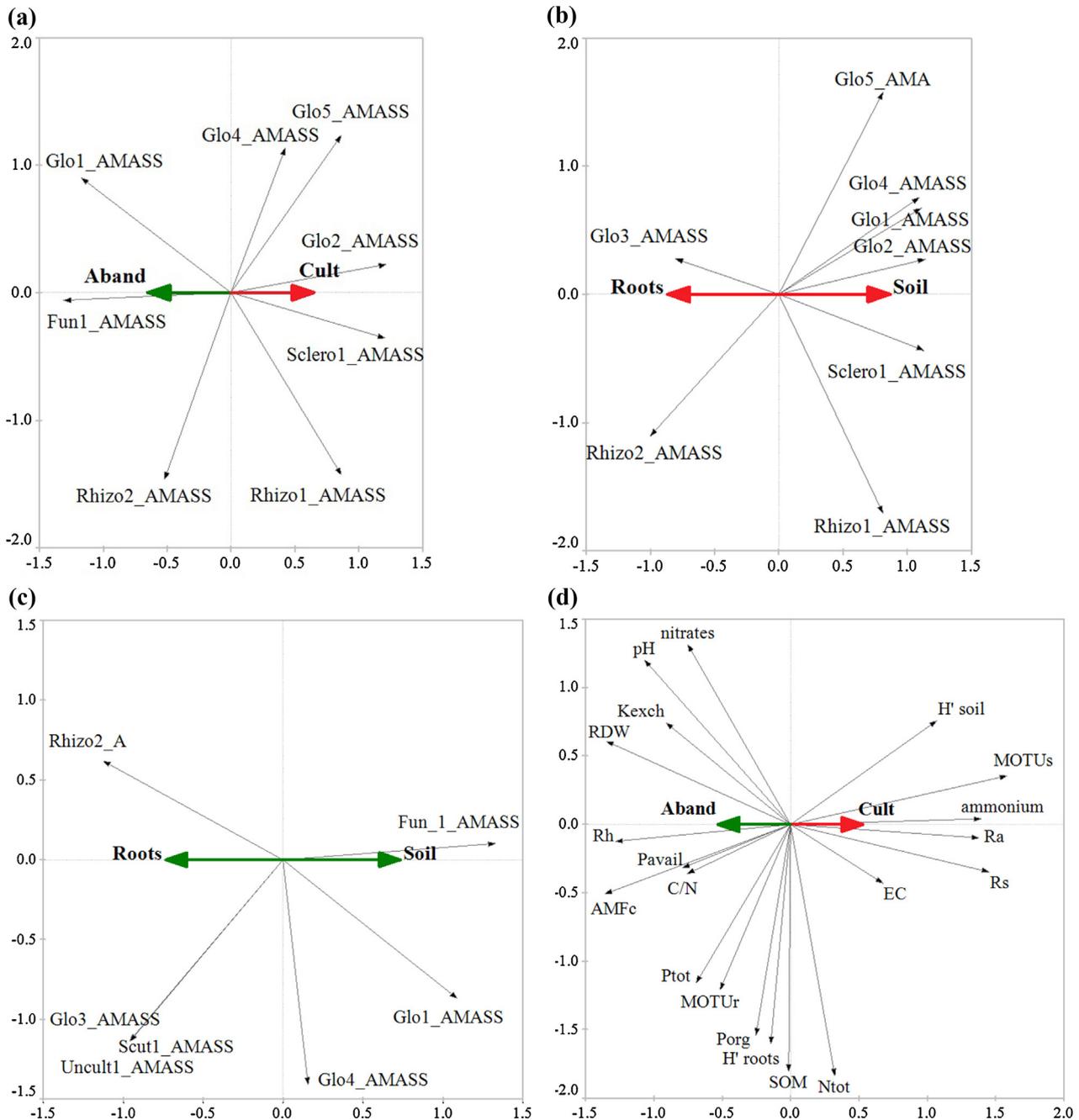


Fig. 2. Partial Redundancy Analysis (PRDA) biplot based on soil relative abundances of arbuscular mycorrhizal fungal (AMF) molecular operational taxonomic units (MOTUs) (*Funneliformis* sp., Fun1_AMASS; *Glomus* spp., from Glo1_AMASS to Glo5_AMASS; *Rhizophagus* spp., Rhizo1_AMASS and Rhizo2_AMASS; *Sclerocystis* sp., Sclero1_AMASS; *Scutellospora* sp., Scut1_AMASS; uncultured Glomeromycota, Uncult1_AMASS) used as response variable, and land uses (agricultural abandoned peaty soils, Aband; a maize cultivation, Cult) used as environmental variables. Plot coordinates (latitude/ longitude) were used as covariables (a). RDA biplots of the AMF MOTUs, used as response variables, and the matrixes, soil and roots, used as environmental variables in the Cult (b) and the Aband (c). pRDA biplot based on soil chemical parameters (pH; electrical conductivity, EC; exchangeable potassium, Kexch; total nitrogen, Ntot; ammonium; nitrates; soil organic matter, SOM; carbon/nitrogen ratio, C/N; total phosphorus, Ptot; available phosphorus, Pavail; organic phosphorus, Porg); CO₂ flux (total soil respiration, Rs; respiration by heterotrophs, Rh; respiration by autotrophs, Ra); root measurements (total root dry weight, RDW; AMF root colonization, AMFc); AMF diversity (AMF MOTU richness of roots and soil, MOTUr and MOTUs, respectively); and Shannon index (H') of roots and soil, H' roots and H' soil, respectively), used as response variables; land uses, Aband and Cult, used as environmental variables. Plot coordinates (latitude/ longitude) were used as covariables (d). The 1st and 2nd axes accounted for 87.2%, 89.7%, 89.2% and 74.7% of the total variance explained by all canonical axes for a, b, c and d, respectively. The Monte Carlo permutational tests showed that AMF assemblages were statistically different between the soil of the Aband and of the Cult and between soil and roots of both Cult and Aband systems ($P=0.036$, a; $P=0.002$, b and c). Land uses were also statistically different considering soil chemical parameters, CO₂ flux, root measurements and AMF diversity ($P=0.05$) (d).

Rh and Ra steadily increased following the trend of air and soil temperatures (Fig. 3c–e). Rs ranged from 19.97 to 54.95 g CO₂ m⁻² day⁻¹ and from 9.74 to 63.35 g CO₂ m⁻² day⁻¹ in the Aband and in the Cult, respectively, while Rh from 17.34 to

35.43 g CO₂ m⁻² day⁻¹ and from 6.79 to 29.12 g CO₂ m⁻² day⁻¹ in the Aband and the Cult, respectively.

The Aband showed significantly higher Rs values in the first period of the monitoring (from the middle to the end of May) than

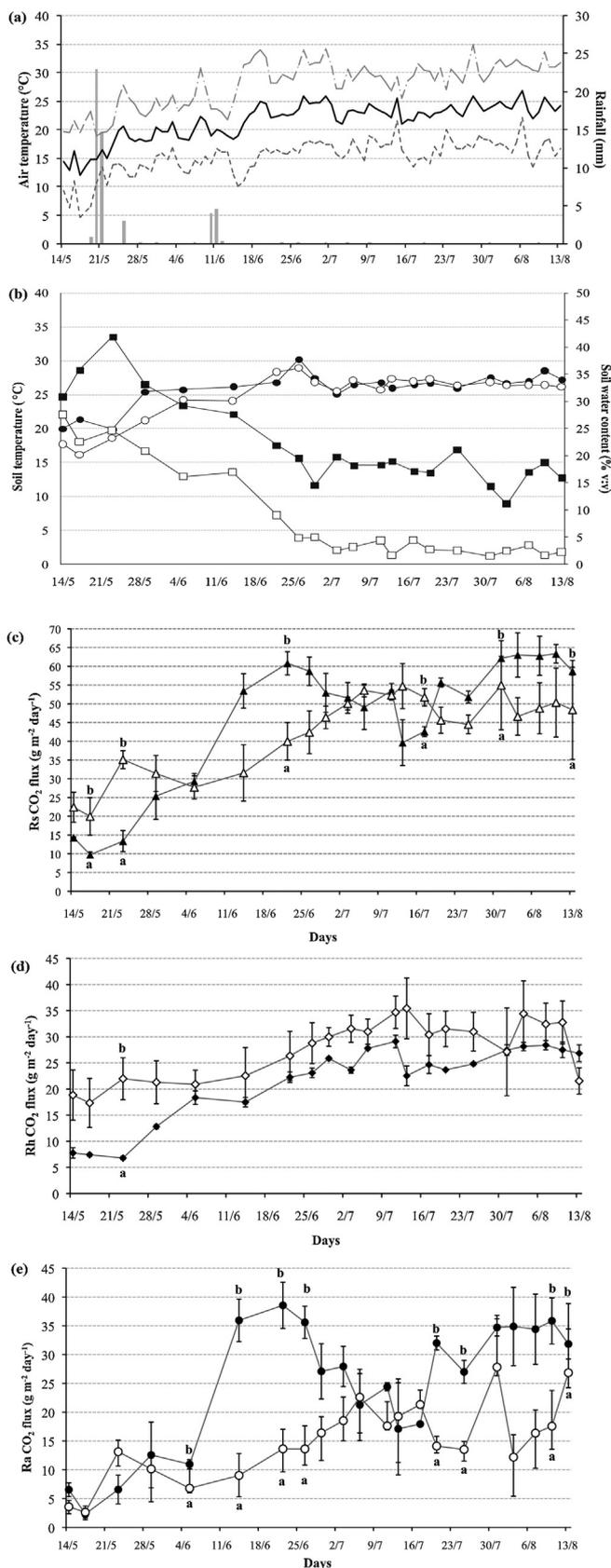


Fig. 3. Daily maximum, mean and minimum temperatures ($^{\circ}\text{C}$) and total rainfall (mm) over the monitoring campaign (a). Mean soil temperature ($^{\circ}\text{C}$) (shown as circle) and moisture (%; v:v) (shown as square) of agricultural abandoned peaty soils (open symbols; Aband) and a maize cultivation (filled symbols; Cult) (b). Soil temperature and moisture were measured outside the six blocks (see Fig. S1) used during the CO_2 flux monitoring campaign. Two components of the soil CO_2 fluxes, respiration by autotrophs (Ra; shown as circle) (c), respiration by heterotrophs (Rh; shown as diamond) (d) were measured in the two different land uses Aband vs Cult (open and filled symbols, respectively). Respiration by autotrophs (Ra; shown as circle) (e) was calculated as difference between Rs and Rh. The monitoring campaign ranges from the 14th of May to the 13th of August 2012 with one or two soil CO_2 flux measurements per week ($n = 21$). Values are means \pm SE of three replicate plots for each land use. For each sampling date and soil CO_2 flux component, statistically significant differences between land uses are shown by different letters according to ANCOVA ($P < 0.05$).

Table 5

Comparison of three models of CO_2 flux response to air temperature using different model selection criteria: the Akaike Information Criterion (AIC), the Root Mean Squared Error (RMSE) and the Adjusted R-square value (Rsq.ad). The measurements ($n = 21$) were recorded from May to August 2012 in abandoned agricultural peaty soils (Aband) and in a maize cultivation (Cult).

Criteria	Q10 [*]		LT		RothC	
	Aband	Cult	Aband	Cult	Aband	Cult
Rs						
AIC	317.9 ^{**}	472.9	317.2	469.9	365.3	531
RMSE	9.1	9.8	9	9.6	16.2	15.9
Rsq.ad	0.5	0.6	0.5	0.7	0.7	0.8
Rh						
AIC	296.6	331.7	296.6	326.4	334.5	384.8
RMSE	7.1	3.2	7.1	3.1	11.3	5
Rsq.ad	0.3	0.8	0.3	0.8	0.7	0.8

^{*} Q10: Van't Hoff's Q10 model; LT: Lloyd and Taylor model; RothC: simplified Rothamsted carbon model.

^{**} Fit model values.

the Cult, whereas, later, we observed an opposite trend. The Ra ranged from 2.63 to 27.82 $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ and from 2.33 to 38.55 $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ in the Aband and the Cult, respectively. Ra in the Cult was significantly higher than in the Aband in the second halves of June and of July, while Rs only in July (Fig. 3c,e).

3.2.2. Model selection of soil CO_2 flux response to air temperature

To calculate the cumulated values of the Rs and Rh of the soil CO_2 respiration, we selected the best of the most commonly used models: Q10, LT and simplified RothC. These models were compared on the basis of AIC, RSME and Rsq. ad (Table 5). The LT model showed the best fit for Rs and Rh in the Aband and the Cult. The LT model minimized the AIC and the RSME values, while the simplified RothC model maximized the Rsq. ad values (Table 5). The LT model was thus selected for modeling the soil CO_2 flux response to air temperature.

The plots of the model fitting are shown in Fig. S7. Clear relationships between Rs and Rh flux and air temperature were observed in both land uses. In the Aband significant relationships from moderate to weak were observed (Rs Rsq. ad = 0.5; Rh Rsq. ad = 0.3; $P < 0.001$) (Table 5; Fig. S7b,d, respectively), while in the Cult strong and significant relationships were revealed (Rs Rsq. ad = 0.7; Rh Rsq. ad = 0.8; $P < 0.001$) (Table 5; Fig. S7a,c, respectively).

3.2.3. Impact of agricultural abandonment on the resilience of CO_2 flux and on cumulated soil CO_2 flux

The coefficients of variation of the Rs and Rh components were significantly lower in the Aband than in the Cult, thus showing a higher fluctuation of soil CO_2 flux response to air temperature (Table 6).

The curves of the Rs, Rh and Ra cumulated CO_2 fluxes are shown in Fig. 4. In the Aband, Rs and Ra cumulated CO_2 flux ($3999 \pm 76 \text{ g m}^{-2} \text{ period}^{-1}$ and $1419 \pm 64 \text{ g m}^{-2} \text{ period}^{-1}$, respectively) was significantly lower than in the Cult ($4360 \pm 43 \text{ g m}^{-2} \text{ period}^{-1}$ and $2316 \pm 51 \text{ g m}^{-2} \text{ period}^{-1}$, respectively) (Fig. 4). Rs and Ra cumulated soil CO_2 flux variations, calculated as ((Aband-Cult)/C-

soil respiration (Rs; shown as triangle) (c), respiration by heterotrophs (Rh; shown as diamond) (d) were measured in the two different land uses Aband vs Cult (open and filled symbols, respectively). Respiration by autotrophs (Ra; shown as circle) (e) was calculated as difference between Rs and Rh. The monitoring campaign ranges from the 14th of May to the 13th of August 2012 with one or two soil CO_2 flux measurements per week ($n = 21$). Values are means \pm SE of three replicate plots for each land use. For each sampling date and soil CO_2 flux component, statistically significant differences between land uses are shown by different letters according to ANCOVA ($P < 0.05$).

Table 6

Coefficient of variation of the total soil respiration (Rs) and of the respiration by heterotrophs (Rh) and by autotrophs (Ra) of two land uses: abandoned agricultural peaty soils (Aband) vs a maize cultivation (Cult) over the monitoring campaign.

Parameter	Coefficient of variation	
	Aband	Cult
CV_Rs (%)	23.3 ^a	32.0 ^b
CV_Rh (%)	19.1 ^a	28.9 ^b
CV_Ra (%)	39.3	36.7

^a Values in the same row followed by different letters are statistically different between land uses according to the Mann–Whitney nonparametric test ($P < 0.05$).

were -8% and -39% , respectively. In contrast, the Rh component showed an opposite behavior with significantly higher values in the Aband ($2580 \pm 80 \text{ g m}^{-2} \text{ period}^{-1}$), than in the Cult ($2045 \pm 8 \text{ g m}^{-2} \text{ period}^{-1}$). In the Cult, the Rh and Ra soil CO_2 flux partitioning was 47% and 53%, respectively, whereas in the Aband it was 65% and 35%, respectively.

3.3. Main patterns of soil chemical parameters, CO_2 flux and AM fungal diversity as affected by agricultural abandonment

pRDA showed that land use explained 74.7% (I and II axes) of the whole variance, and that its effect on soil quality parameters was significant ($P = 0.05$), as shown by the biplot arrows representing the Aband and the Cult (Fig. 2d). The biplot clearly suggested that the most discriminant variables between the two land uses were NH_4^+ , soil AMF MOTU richness, Ra and Rs, which showed lower values in the Aband compared to the Cult, and Rh and AM fungal colonization, which showed higher values in the Aband than the Cult.

4. Discussion

In this work we assessed for the first time the long-term effect on soil quality of agricultural abandonment in a Mediterranean reclaimed peaty soil. Agricultural abandonment (Aband) was compared to an intensively cultivated peaty soil represented by continuous maize (Cult). Multivariate analyses showed that 15 years of agricultural abandonment: (i) did not affect the main soil chemical parameters, except for NH_4^+ which was lower in the Aband than in the Cult; (ii) increased AM fungal root colonization and root AM fungal taxonomic diversity, but decreased soil MOTU richness; (iii) reduced soil and root respiration together with an

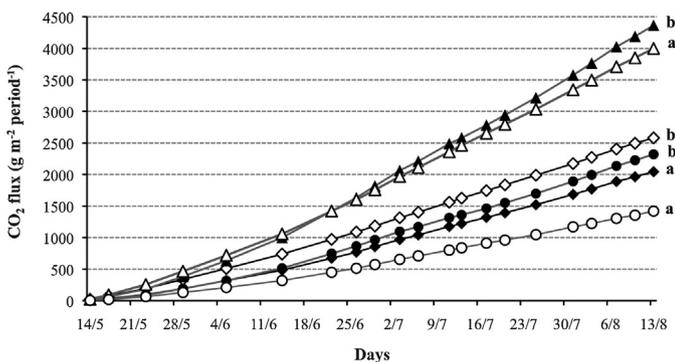


Fig. 4. Cumulated soil CO_2 flux: soil respiration, Rs (shown as triangle); respiration by heterotrophs, Rh (shown as diamond) and respiration by autotrophs, Ra (shown as circle). The measurements ($n = 21$) were recorded from May to August 2012 in agricultural abandoned peaty soils (Aband; open symbols) and a maize cultivation (Cult; filled symbols). Values are means of three replicate plots for each land use. Statistically significant differences between land uses are shown by different letters according to the ANCOVA ($P < 0.05$).

increase in respiration by heterotrophs; (iv) increased soil resilience to air temperature in terms of CO_2 flux response.

4.1. Effect of agricultural abandonment on soil chemical parameters, roots and AM fungi (Experiment 1)

4.1.1. Soil chemical parameters

To the best of our knowledge, our work is the first one to report on the impact of the abandonment of the agricultural practices in organic drained soil. However, our results confirmed previous data detected in mineral abandoned soils which, compared to conventional farming, showed no changes in the main chemical aspects of soil quality due to fallow, land abandonment or grassland reestablishment (Liebig et al., 2004; Pellegrino et al., 2011; Bell et al., 2012).

On the other hand, our findings are in contrast with other studies reporting negative changes due to land use intensification in comparison with agricultural abandonment or grassland (Celik, 2005; Marriott et al., 2010; Gajić, 2013). These inconsistencies could be attributed to the sampling depth and to the time elapsed since land-use conversion (Liebig et al., 2004; Jinbo et al., 2007; Raiesi, 2012; Gajić, 2013). In fact differences were observed only at a 0–15 cm depth and already six years after abandonment. Interestingly, in line with our results, Ewing et al. (2012) found no significant changes in organic soils of total organic carbon after 15 years of crop production in reclaimed wetlands in comparison with natural wetland soils.

The fact that the only difference observed was in the soil NH_4^+ concentration could be explained by the high temporal variability of such a chemical compound (Violante, 2000) or by the closeness of the sampling to the N fertilization in the Cult.

Concerning the C/N ratio, our data are in the value range reported for highly-decomposed cultivated peaty soils (Berglund et al., 2010).

4.1.2. Root dry weight and AM fungal root colonization

The root dry weight was in the range of those obtained for uncultivated natural fallow and maize ($0.04\text{--}1.8 \text{ mg g}^{-1}$) (Kothari et al., 1990; Mekonnen et al., 1997). Despite the well-known highly mycotrophic status of maize (Gavito and Varela, 1993), in our study the AM fungal root colonization of maize was lower than the natural plant species occurring in the Aband. This cannot be explained by the plant species composition in the Aband, since most of the plant species is known to be a non-mycorrhizal status (Harley and Harley, 1987). Therefore the difference is very likely due to the tillage, fertilization, mechanical and chemical weeding carried out in the Cult (Helgason et al., 1998; Vandenkoornhuys et al., 2002; Borriello et al., 2012).

4.1.3. AM fungal diversity

Although in the past the importance of AMF in wetlands was not taken into account, our study on the AM fungal diversity of a Mediterranean reclaimed peaty soils was boosted by the increased awareness of their occurrence and functionality in these key ecosystems (Wolfe et al., 2007).

Root and soil MOTU richness and H' fell into a similar range to previous works (Helgason et al., 1998; Daniell et al., 2001; Hijri et al., 2006; Borriello et al., 2012), but were mostly lower than those reported by other authors (Jansa et al., 2002; Wolfe et al., 2007; Oehl et al., 2010). These inconsistencies might be due to the pedo-climatic dissimilarities, but also to the differences in detection methodologies. Root MOTU richness and H' suggested that the AM fungal diversity was not depleted by the intensification of cultivation, in line with findings in field and microcosms (Daniell et al., 2001; Johnson et al., 2004; Hijri et al., 2006). The

higher MOTU richness in the Cult soil than in the Aband soil may be due to weed mycotrophic composition (Poaceae, Solanaceae and Phytolaccaceae).

Glomeraceae was the only family retrieved in the soil, while a member of Gigasporaceae was also detected within the roots. Our data are consistent with previous observations: Glomeraceae are mainly retrieved in agricultural soil and wetlands, while Gigasporaceae are more frequent in uncultivated or woodland sites (Helgason et al., 1998; Daniell et al., 2001; Jansa et al., 2002; Wirsal, 2004; Wolfe et al., 2007).

Within Glomeraceae, the dominance of the Rhizo2_AMASS within maize roots suggests that it can survive in agricultural conditions due to its ability to colonize the roots from mycelium fragments (Biermann and Linderman, 1983). The presence within the roots of the natural plant species of a MOTU that is phylogenetically affiliated to *Scutellospora* sp. is likely due to the lack of soil tillage disruption of the extraradical hyphae, which is essential for the propagation of Gigasporaceae (Jasper et al., 1993). Despite several authors highlighting the dominance of *Funnelformis mosseae* in arable land, grassland and also wetlands, we unexpectedly retrieved *Funnelformis* sp. only in the soil of the Aband, and it was not the most representative species.

4.2. Effect of agricultural abandonment on soil CO₂ flux (Experiment 2)

4.2.1. Soil CO₂ flux measurements and cumulated values

Differentiating the soil CO₂ flux into its autotrophic and heterotrophic components is important for interpreting soil CO₂ sources (Baggs, 2006; Kuzyakov, 2006). In our study, the soil CO₂ sources were successfully monitored in abandoned and cultivated peaty soils using the root exclusion method. In addition, in order to fill respiration gaps and to assess the total respiration over the monitored period, the air temperature was used as an environmental variable for CO₂ modeling. In fact, although some authors have modeled soil respiration using environmental parameters, such as soil water content and water table depth (Almagro et al., 2009; Correia et al., 2012; Rowson et al., 2013), most authors have found a highly significant relationship between soil or air temperature and soil respiration (Luo and Zhou, 2006; Richardson et al., 2006; Subke et al., 2006). On the basis of three different criteria, we thus selected the LT model in terms of the Q10 and the simplified RothC, in agreement also with Davidson et al. (2006) and Luo and Zhou (2006).

We used the cumulated values of the Rs, extrapolated using the LT model, to obtain the means of the daily Rs values. The calculated daily Rs fluxes of the Aband and the Cult over the monitoring period (43.47 and 47.39 g CO₂ m⁻² day⁻¹, respectively) were two-fold higher than reported by several authors as annual mean daily values (from 0.77 to 26.6 g CO₂ m⁻² day⁻¹) for fens and peatlands in boreal and temperate areas (Silvola et al., 1996; Kasimir-Klemedtsson et al., 1997; Danevčič et al., 2010; Heinemeyer et al., 2011). This large fluxes could be explained by the fact that we monitored the spring–summer period, characterized by a warmer average temperature (21.5 °C) than the annual average temperature (14.8 °C). Although this estimation needs to be confirmed by a long-term monitoring, a strong mineralization rate is pointed out in Mediterranean peaty soils subjected to agricultural reclamation, implying a large loss of SOC and soil depth.

In addition to such factors, the not limiting soil water content could have determined larger fluxes compared to those commonly registered in mineral soils in Mediterranean areas (from 3.19 to 25.09 g CO₂ m⁻² day⁻¹) (Almagro et al., 2009; Mancinelli et al., 2010; Correia et al., 2012).

With regard to the partitioning, the two respiration components showed an opposite performance since the heterotrophic component was predominant in the Aband and the autotrophic

component in the Cult. An explanation for the significantly higher daily values of the Ra component in the Cult is to be found in the boost of the fine roots during the growing season of the maize crop (Rochette and Flanagan, 1997; Werth and Kuzyakov, 2009). Such effect results in a progressive increase of the flux from the sowing (first part of May) to the crop development (mid and late summer).

As suggested by Subke et al. (2006), we also compared our results on soil CO₂ partitioning using the Rh/Rs ratio. The Rh/Rs ratio ranged from 0.65 in the Aband, with values similar to the pristine peatland to 0.47 the Cult, similar to other croplands values. These ratios were similar to those reported in Mediterranean croplands, where the mean ratio was around 0.50 (Subke et al., 2006). In contrast, our ratios were lower than those reported for boreal and temperate peatlands, ranging from 0.72 to 0.97 (Silvola et al., 1996; Wunderlich and Borken, 2012).

When considering values coming from root exclusion methods it is important to take into account the bias that this method implies. It is known that the major concern associated with this technique results in an increase in the dead root biomass in the deep collar (compared to the shallow one) that contribute to Rh and lead to an underestimation of Ra (Baggs, 2006; Subke et al., 2006; Heinemeyer et al., 2007). Moreover, some authors highlighted direct correlations between Rs and productivity and proposed that in more productive systems, as the case of Mediterranean peaty soils compared to boreal peatlands, a greater amount of assimilated C is allocated to Ra, thus reducing Rh/Rs ratio (Raich and Tufekciogul, 2000; Subke et al., 2006). Finally, another factor to take into consideration when comparing our ratio values to other ecosystems is that we monitored the spring–summer period when the Ra component, following the typical annual phenology of the vegetation, is at its maximum (Silvola et al., 1996).

4.2.2. Resilience of the CO₂ fluxes

In agreement with our data, comparing the fluctuation of the CO₂ flux in the Aband compared to the Cult, Jackson et al. (2003) reported a lower resilience in the tilled cropping systems than in the non-tilled ones. This could be explained by the higher porosity, the initial lower bulk density and the lower pore connectivity of the long-term tilled soils compared to the non-tilled soils (Silgram and Shepherd, 1999). Laliberté et al. (2010) also linked the reduction in resilience following land use intensification to plant diversity and functionality. Together with this, our maize system also showed lower plant diversity than in the abandoned peaty soil.

5. Conclusions

Our analysis of the chemical changes and of the peat mineralization measured using respiration by heterotrophs revealed 15 years of agricultural abandonment does not necessarily lead to the effective restoration of a Mediterranean reclaimed peatland in terms of the soil quality. The large CO₂ flux that we observed demonstrates the strong degradation rate of peat—whether cultivated or abandoned—and the importance of this flux as a diffuse source of CO₂ at the landscape scale. As a consequence, it highlights a strong mineralization rate in Mediterranean peaty soils subjected to reclamation, implying an important loss in SOC and in soil depth.

However, there were some positive effects in the abandonment: an increase of the diversity in terms of number of families of AMF retrieved in roots, a small reduction of total soil CO₂ respiration and a lower fluctuation of soil CO₂ flux response to air temperature.

We believe that our findings can be used to better understand on how best to protect and preserve the Mediterranean peatlands. They can help to develop alternative and sustainable solutions for their restoration, supporting the importance of

hydrological conditions (e.g., rewetting) as a major factor to trigger the restoration and to limit the mineralization rate thus controlling the subsidence effect.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2014.09.004>.

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