

1 **NEW INSIGHTS INTO MEDITERRANEAN PEATLANDS: LONG-TERM**  
2 **EFFECT OF CONVENTIONAL CONTINUOUS MAIZE ON SOIL QUALITY**  
3 **ASSESSED BY PHYSICO-CHEMICAL PARAMETERS, ARBUSCULAR**  
4 **MYCORRHIZAL FUNGAL DIVERSITY AND *IN-SITU* CO<sub>2</sub> FLUXES**

5

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17

## 18 **Abstract**

19 Wetlands are species-rich habitats performing valuable ecosystem services such as  
20 flood protection, water quality enhancement and carbon sequestration, therefore their  
21 protection is a priority. These habitats cover about 6% of the global surface area and  
22 about 60% are represented by peatlands. In Europe, peatlands cover about 20% of the  
23 land area and, although most are located in the Baltic Sea basin, some narrow sites are  
24 situated also in the Mediterranean area. So far, despite several studies have been  
25 performed on the soil quality changes of peatlands, due to land use or management  
26 change, using physico-chemical parameters, up until now there is still a lack of  
27 information regarding the effects on the biological and biochemical parameters with  
28 specific regards to Mediterranean peatlands. In the present study, we evaluated the long-  
29 term effect of a conventional continuous maize (ConvMaize) on the soil quality of a  
30 drained Mediterranean peatland. This effect was compared to an ex-arable cropping  
31 land being uncultivated for 15 years (Aband). The main soil physico-chemical  
32 parameters, arbuscular mycorrhizal (AM) fungal molecular richness, composition and  
33 structure and *in situ* soil respiration partitioning were assessed. In contrast with  
34 expectations, the main soil physico-chemical parameters of the ConvMaize did not  
35 significantly differ respect to the Aband. As regard AM fungal community diversity, 11  
36 different AM fungal molecular operational taxonomic units affiliated to *Funneliformis*  
37 *mosseae*, 5 different *Glomus* spp., *Rhizophagus manihotis*, *Rhizophagus irregularis*,  
38 *Sclerocystis sinuosa*, *Scutellospora dipurpurescens* and an uncultured Glomeromycota,  
39 were retrieved. Multivariate analysis showed that the ConvMaize significantly affected  
40 soil AM fungal communities and highlighted the presence of preferential and ubiquitous  
41 AM fungi. The cumulated values of soil CO<sub>2</sub> flux components were modeled using the  
42 selected Lloyd and Taylor equation. In the ConvMaize total and autotrophic respiration

43 cumulated CO<sub>2</sub> fluxes were significantly higher than in the Aband (9% and 63%,  
44 respectively). By contrast, the heterotrophic respiration component showed an opposite  
45 behavior (- 21%). In addition, the ConvMaize reduced the soil resilience to the air  
46 temperature changes in terms of soil CO<sub>2</sub> flux response. These findings enhance our  
47 knowledge on the biological and biochemical changes due to land use change of  
48 peatlands and can be used to protect and preserve the Mediterranean peatlands and to  
49 identify sustainable solutions for their management.

50

51 *Keywords:* peatland, sustainability, land use change, arbuscular mycorrhizal fungi  
52 (AMF), AM fungal diversity, soil CO<sub>2</sub> flux, soil respiration partitioning, heterotrophic  
53 respiration

54

## 55 **1. Introduction**

56 Wetlands are species-rich habitats performing valuable ecosystem services such as  
57 flood protection, water quality enhancement and carbon (C) sequestration and their  
58 protection is officially a priority for 168 nations that have ratified the Ramsar  
59 Convention (Verhoeven and Setter, 2010). These habitats cover about 6% of the global  
60 surface area and about 60% are represented by peatlands, which play an important role  
61 in global C cycle as a long-term sink (Clymo, 1984). In Europe peatlands cover about  
62 20% of the land area and, although most are located in the Baltic Sea basin, some  
63 narrow sites are situated also in the Mediterranean area (Montanarella et al., 2006).  
64 Theneglectful management of these fundamental ecosystems, including their drainage  
65 for agricultural purposes, can lead to problems such as soil organic matter (SOM)  
66 oxidation with severe carbon dioxide (CO<sub>2</sub>) emissions, subsidence, nutrient losses to  
67 water bodies, biodiversity losses and decrease of soil quality (Foley et al., 2005;  
68 Tiemeyer et al., 2007; Verhoeven and Setter, 2010).

69 As defined by Doran and Parkin (1994) soil quality is “the ability of soils to interact  
70 with the ecosystems in order to maintain the biological productivity, the environmental  
71 quality and to promote animal and vegetal health”. Soil quality may be affected by  
72 environmental unfriendly land use and agricultural management practices because these  
73 may cause alterations of the soil physico-chemical, biochemical and biological  
74 properties (Lal, 1998; Schjøning et al., 2004) determining, in turn, a reduction of  
75 cropping productivity and sustainability and of the ecosystems services provided by the  
76 soil (Lal, 1998). As soil quality cannot be measured directly, indicators are assessed and  
77 evaluated for their suitability (Schloter et al., 2003). Adequate soil quality indicators  
78 should be sensitive, robust, accurate, precise, simple to be measured, work equally well  
79 in all environments, user-friendly and cost-effective (Doran and Parkin, 1994; Karlen et

80 al., 1997). Within the most suitable and suggested set of soil physico-chemical,  
81 biochemical and biological indicators, the soil organic carbon (SOC) is one of the main  
82 chemical indicators influencing aggregation, nutrient availability, C storage and  
83 biodiversity (Baldock and Skjemstad, 2000).

84 As regards biochemical parameters, microbial biomass and soil respiration are the two  
85 main indicators and are used for their rapidity of reaction to environmental changes and  
86 reproducibility (Bloem et al., 2006). However, the *ex situ* measurements have  
87 limitations since they are assessed in defined laboratory conditions and cannot  
88 distinguish below-ground sources of soil respiration (Rs) and their individual responses  
89 to key environmental factors (Bloem et al., 2006). Rs is in fact a complex flux, resulting  
90 from the metabolic activity of plant roots (autotrophic respiration: Ra) and soil  
91 microorganisms such as bacteria, non-mycorrhizal and mycorrhizal fungi,  
92 actinomycetes and mesofauna (heterotrophic respiration, Rh) (Kuzyakov, 2006).  
93 Therefore, *in situ* measurements of CO<sub>2</sub> fluxes, using root exclusion, shading and  
94 clipping, tree girdling or isotopic techniques, have been largely used because of their  
95 capacity to distinguish soil respiration components (Hanson et al., 2000; Luo and Zhou,  
96 2006; Heinemeyer et al., 2007). Moreover, the selection of an appropriate model for soil  
97 respiration is important for an accurate CO<sub>2</sub> flux gap filling (Richardson et al., 2006).  
98 Numerous empirical models have been devised and validated which are mostly simple  
99 functions of few independent variables and several parameters (Luo and Zhou, 2006).  
100 So far, in simple models the Rs has been scaled with air and soil temperature and  
101 showed exponential relationships.

102 Within biological indicators, arbuscular mycorrhizal (AM) fungal diversity has been  
103 shown to be a good and suitable biological indicator of the state and functioning of  
104 ecosystems. Arbuscular mycorrhizal fungi (AMF, phylum: Glomeromycota, Schüßler et

105 al. 2001) are one of the main components of the soil microbiota in most natural and  
106 agricultural ecosystems. They form mutualistic symbioses with the roots of most land  
107 plants and they play fundamental roles since they increase plant growth, enhance nutrient  
108 uptake, in exchange of photosynthetically fixed C (Bago et al. 2000), improve soil  
109 structure and protect against biotic and abiotic stressors (Smith and Read, 2008).  
110 However, several studies have shown that AM fungal diversity and their benefits are  
111 affected by land use change and agricultural system intensification (Moora et al., 2007;  
112 Oehl et al., 2010; Verbruggen and Kiers, 2010; Pellegrino et al., 2011; Davison et al.,  
113 2012). In fact, the intensification of land use, tillage and application of fertilizers and  
114 biocides together with cropping sequence choices (i.e., rotations with non-mycorrhizal  
115 hosts) have been shown to negatively modify AM fungal community composition and  
116 structure and soil aggregation (Helgason et al., 1998, 2007; Jansa et al., 2002; Oehl et  
117 al., 2010; Rillig et al., 2003; Rillig and Mummey, 2006; Borriello et al., 2012).

118 So far, despite several studies have been performed on the soil quality changes of  
119 peatlands, due to land use or management change, using the main physico-chemical  
120 parameters (Tiemeyer et al., 2007; Pulleman et al., 2010), up until now there is still a  
121 lack of information regarding the effects on the biological and biochemical parameters.  
122 These studies are indeed lacking in Mediterranean peatlands and need to be included in  
123 conservation and preservation programs, as strongly suggested by the Convention for  
124 Biological Diversity (Sutherland et al., 2009).

125 In the present study, starting from the lack of information on the influence of land use  
126 change on soil organic matter degradation, CO<sub>2</sub> flux and AM fungal diversity in  
127 Mediterranean peatlands, we evaluated the long-term effect of a conventional  
128 continuous maize in comparison to an ex-arable cropping land, being uncultivated for  
129 15 years, on the soil quality of a drained peatland in the Massaciuccoli Lake basin

130 (Tuscany, Italy). The continuous maize was chosen since it is the most widespread  
131 cropping system in the lake basin (about 30% of the total UAA; Silvestri et al. 2012).  
132 The main soil physico-chemical parameters, AM fungal molecular richness,  
133 composition and structure and *in situ* soil respiration partitioning (Ra and Rh) were  
134 utilized for this evaluation. In addition, a model selection for soil respiration was  
135 performed in order to calculate the cumulative respiration fluxes.

136

## 137 **2. Materials and Methods**

### 138 *2.1. Field site*

139 The experimental site was situated in the southern part of the Massaciuccoli Lake  
140 basin (43°49'N - 10°19'E) within the Regional Park of Migliarino-San Rossore-  
141 Massaciuccoli (Pisa, Italy). Since 1930, most of the basin has been drained by a  
142 complex network of artificial canals and ditches and pumping stations, forcing water  
143 from reclaimed areas into the lake, causing a severe subsidence ranging from 3 to 4 cm  
144 yr<sup>-1</sup>, determined by the compaction and the peat oxidation (Pistocchi et al., 2012). The  
145 soil was classified as *Histosol* according to the USDA system (Soil Survey Staff, 1975)  
146 and as *Rheic Histosol* according to the FAO system (IUSS, 2006). The soil is classified  
147 as a peat-topped soil (peatland) according to Montanarella et al. (2006) and as a sandy  
148 clay loam (50.0% sand, 24.8% silt and 20.6% clay) with a mean values of SOM equal to  
149 29.2% [minimum 20.1% - maximum 55.4% (Pistocchi et al., 2012)] (Walkley-Black),  
150 pH of 4.9 and the following total nutrient concentrations: 12.9 g kg<sup>-1</sup> N (Kjeldahl),  
151 2896.4 mg kg<sup>-1</sup> P and 75.2 mg kg<sup>-1</sup> available P (Olsen). During the year the water table  
152 depth is maintained at a quite stable level, ranging from 0.40 to 0.60 m by the pumping  
153 stations (Rossetto R., personal communication). Climate at the experimental station is  
154 Mediterranean (Csa) according to the Köppen Geiger climate classification map.

155 Summers are dry and hot, rainfall is mainly concentrated in autumn and spring (mean  
156 annual rainfall ca. 900 mm year<sup>-1</sup>) and mean monthly air temperature ranges from 7°C  
157 in February to 30 °C in August (mean of 14.5 °C year<sup>-1</sup>). Average monthly maximum,  
158 mean and minimum temperatures and rainfalls were recorded at the weather station of  
159 Metato (Pisa, Italy; 43°77'N - 10°38'E) in the period 1989-2012 (Fig. S1).

160

## 161 *2.2. Experiment 1: evaluation of the impact of land use on soil physico-chemical* 162 *parameters and AMF*

163

164 This experiment aimed at evaluating the long-term effect of a conventional continuous  
165 maize system in comparison with land abandonment on physico-chemical soil  
166 parameters, root traits, such as the root biomass and the AM fungal colonization, and  
167 AM fungal diversity, composition and structure.

168

### 169 *2.2.1. Experimental set-up*

170

171 The experiment was a completely randomized design with land use as treatment and  
172 three replicates (n = 3; replicate plots of 7000 m<sup>2</sup>). Land use types were:  
173 (1) a conventional continuous maize (*Zea mays* L.) (ConvMaize). Each year at late  
174 spring plots were deeply ploughed (30-35 cm) and harrowed as main and secondary  
175 tillage, respectively. Maize was sown at the beginning of June at a rate of 75,000 seeds  
176 ha<sup>-1</sup> with 75 x 17 cm row spacing and harvested on late September. Fertilization was  
177 applied at sowing and at mechanical weeding with rates of 32 kg ha<sup>-1</sup> N, 96 kg ha<sup>-1</sup> P,  
178 96 kg ha<sup>-1</sup> K and 138 kg ha<sup>-1</sup> N, respectively. Chemical and mechanical post-emergence  
179 weed controls were applied. Average maize yield of the last 15 years was 6.4 tha<sup>-1</sup>;

180 (2) an ex-arable cropping land, being uncultivated for 15 years (Aband). Plots were left  
181 to develop under the natural succession vegetation. A floristic survey carried out in  
182 summer 2011 showed that the most common species were *Abutilon theophrasti* L.,  
183 *Amaranthus retroflexus* L., *Arctiumlappa* L., *Artemisia* sp., *Atriplex* sp., *Bidens* sp.,  
184 *Biphora* sp., *Calystegia* sp., *Datura stramonium* L., *Echinochloa crus-galli* L., *Galium*  
185 sp., *Humulus lupulus* L., *Linaria* sp., *Phragmites australis* L, *Typha latifolia* L.,  
186 *Lythrum salicaria* L., *Phytolacca americana* L., *Rumex crispus* L., *Silene alba* L. and  
187 *Xanthium* sp.. No fertilizers or other agricultural practices were applied, except for an  
188 annual vegetation cutting. Details of soil texture of the two land uses (ConvMaize and  
189 Aband) are given in Table 1.

190

### 191 2.2.2. Sampling

192

193 In July 2011 one combined soil sample, resulting from pooling three soil cores, was  
194 collected from each replicate plot (30 cm depth) in order to control physico-chemical  
195 and AM fungal spatial variability. Sampling was carried out only once in July since  
196 mid-summer is the best choice according to the fact that sampling must not be close to  
197 soil treatments and the variability of physico-chemical parameters slightly change  
198 during the year (Pellegrino et al., 2011). These facts, along with the fact that AMF  
199 among differently managed systems consistently maintain the same patterns of  
200 variability although showing seasonal changes (Vandenkoornhuyse et al., 2002; Oehl et  
201 al., 2010; Pellegrino et al., 2011; Di Bene et al., 2013), were taken into strong account  
202 for choosing as a single sampling time July.

203 Soil samples used for the physico-chemical parameter determinations were oven dried  
204 at 60 °C and sieved at 2 mm, while for genomic DNA extraction were stored at 4 °C  
205 after sieving. As regard to the root traits determinations, three turfs were extracted (20

206 cm depth) from each replicate plot and then combined. In laboratory, from each  
207 combined sample, roots were collected, washed and dried at 60 °C for root dry weight  
208 (DW) measurement, whereas for AM fungal root colonization assessment and genomic  
209 DNA extraction, root subsamples were taken and stored at 4 °C.

210

### 211 *2.2.3. Soil physico-chemical analyses*

212

213 Soil samples were analyzed for: pH; electrical conductivity, EC; exchangeable  
214 potassium,  $K_{\text{exch}}$ ; total nitrogen,  $N_{\text{tot}}$ ; ammonium,  $\text{NH}_4^+$ ; nitrates,  $\text{NO}_3^-$ ; soil organic  
215 matter, SOM; total phosphorus,  $P_{\text{tot}}$ ; available phosphorus,  $P_{\text{avail}}$  and organic phosphorus,  
216  $P_{\text{org}}$ . Soil pH and EC were measured in deionized water (1:2.5 and 1:2, w/v,  
217 respectively) (McLean, 1982).  $K_{\text{exch}}$  was determined after the atomic absorption  
218 (Thomas, 1982).  $P_{\text{tot}}$  and  $P_{\text{avail}}$  were determined by colorimetry using perchloric acid  
219 digestion and a solution of sodium bicarbonate, respectively (Olsen and Sommers,  
220 1982).  $P_{\text{org}}$  was evaluated using the Metha extraction (Hence and Anderson, 1962).  $N_{\text{tot}}$   
221 was determined by the macro Kjeldahl digestion procedure (Bremner and Mulvaney,  
222 1982), while  $\text{NO}_3^-$  and  $\text{NH}_4^+$  by the Keeney and Nelson method (1982). SOM was  
223 measured using the modified Walkley-Black wet combustion method (Nelson and  
224 Sommers, 1982). Soil C/N ratio was calculated dividing SOC ((SOM / 1.7) x 10) by  
225 total N.

226

### 227 *2.2.4. Root determination and AM fungal root colonization*

228

229 From the combined turfs of each replicate plot, soil subsamples (mean soil DW ca.  
230 400 g) were used to determine root DW. Roots were manually collected with forceps  
231 and washed by wet-sieving and decanting down to a mesh size of 250  $\mu\text{m}$ . After

232 removing organic debris, all live and dead root fragments were oven-dried and weighed  
233 to determine root DW. Root DW per gram of soil was calculated by dividing root DW  
234 for soil DW.

235 AM fungal root colonization was assessed under a stereomicroscope (Olympus SZX  
236 9, Olympus Optics, Tokyo, Japan), after clearing and staining with lactic acid instead of  
237 phenol (Phillips and Hayman, 1970), following the gridline intersect method  
238 (McGonigle et al., 1990).

239

240 *2.2.5. AM fungal diversity: extraction of genomic DNA, PCR amplification, cloning and*  
241 *sequencing*

242

243 Soil DNA was extracted from 0.5 g of soil using the PowerSoil<sup>®</sup>MoBio kit (Mo Bio  
244 Laboratories Inc., NY, USA) (n = 6), while root DNA from 100-mg fresh root samples  
245 using the DNeasy<sup>®</sup> Plant Mini Kit (n = 6) (Qiagen, Germantown, MD, USA). DNA was  
246 stored at -20 °C until PCR amplification. PCR amplification was performed using the  
247 primers pair NS31 and AM1 targeting the small subunit ribosomal RNA (SSU rRNA)  
248 region (Simon et al., 1992; Helgason et al., 1998). The NS31/AM1 SSU region,  
249 although the availability of long and highly discriminate regions (Krüger et al., 2009;  
250 Pellegrino et al., 2012), was targeted because most data of Glomeromycota diversity are  
251 obtained using it, providing a larger comparative DNA sequence data-set than other  
252 obtained using other genomic regions. PCR was performed using the temperature  
253 profile described by Helgason et al. (1998). PCR amplicons were generated from 10 ng  
254  $\mu\text{L}^{-1}$  genomic DNA in volumes of 20  $\mu\text{L}$  with 0.5 U of GoTaq<sup>®</sup> Hot Start Polymerase  
255 (Promega Corporation, Madison, WA, USA), 0.2  $\mu\text{M}$  of each primer (NS31/AM1), 0.2  
256 mM of each dNTP, 1.25 mM of  $\text{MgCl}_2$  and 1x reaction buffer, using the S1000 Thermal  
257 Cycler<sup>™</sup> (BIORAD, USA). Before ligation, quantity and quality of the PCR amplicons

258 were checked by a spectrophotometer (NanoDrop®ND-1000, Germany). The PCR  
259 amplicons were then ligated into the pGem®-T Easy vector (Promega Corporation,  
260 Madison, WA, USA) and used to transform XL10-Gold® Ultracompetent *Escherichia*  
261 *coli* cells (Stratagene®, La Jolla, CA, USA). At least 25 recombinant clones per  
262 amplicon library (n = 12) were screened for the c. 550-bp-long NS31/AM1 fragment  
263 (Helgason et al., 1998) on agarose gels. A total of 270 PCR products obtained from  
264 clones (a mean of 23 per library) were sequenced using the NS31/AM1 primers in an  
265 ABI Prism® 3730XL automated sequencer (Applied Biosystem, Foster City, CA, USA)  
266 at the High-Throughput Genomics Unit (Seattle, WA, USA).

267

### 268 *2.3. Experiment 2: evaluation of the impact of land use on soil CO<sub>2</sub> fluxes*

269

270 This experiment aimed at evaluating the long-term effect of a conventional maize  
271 system in comparison with land abandonment on total soil respiration flux and  
272 autotrophic and heterotrophic CO<sub>2</sub> flux components.

273

#### 274 *2.3.1. Experimental set-up and sampling*

275 The experimental design was as described above for experiment 1. Six experimental  
276 blocks (one for each replicate plot) were established within the area for soil respiration  
277 monitoring (Fig. S2a,b). Six blocks were considered an adequate number due to the soil  
278 texture homogeneity. In each plot the block was composed by two 20-cm diameter  
279 open-ended PVC collars (Plumb Centre, Pisa, IT; Toscana Tubi Srl, Livorno, IT): a  
280 surface collars (7.0-cm deep collar inserted 1 cm into the soil) pressed firmly onto the  
281 shallow surface layer without cutting any roots (Fig. S2a); a 25-cm deep collar tapered  
282 on the bottom inserted 20 cm into the soil (Fig. S2b). Plants inside the collars were  
283 removed, leaving the root systems intact, while litter was maintained. The surface and

284 the deep collars provided a measure of total soil respiration ( $R_s$ ) and heterotrophic  
285 respiration ( $R_h$ , soil microorganisms and mesofauna), respectively (Heinemeyer et al.,  
286 2007). These measures were utilized to calculate the contribution of the root component  
287 defined as autotrophic respiration ( $R_a = R_s - R_h$ ). Soil  $CO_2$  flux was measured *in situ*  
288 using the manual dynamic chamber method, with a portable infrared gas analyzer  
289 (IRGA) (Licor LI-820) connected to a steel accumulation chamber having a headspace  
290 volume of 6186 cm<sup>3</sup> (chamber B, West Systems Srl, Pontedera, Italy). To guarantee a  
291 tight seal with the collars, the chamber has a rubber ring that fits into the collar lip. The  
292  $CO_2$  flux was checked for linearity over a period of 2-3 min and then recorded by a  
293 portable device connected by a wireless Bluetooth connection. An internal fan allowed  
294 the homogeneity of the air mixture inside the chamber during the measurement. Soil  
295 moisture and temperature were recorded at each measurement next to each collar by a  
296 probe (Decagon Devices ECH<sub>2</sub>O-TE/EC-TM) inserted into a soil depth of 5 cm.

297 In 2012 a monitoring campaign was undertaken between 14 May and 13 August with  
298 a measurement frequency of one or two times per week (a total of 21 measurements).  
299 The sampling period was chosen taking into consideration that in order to evaluate the  
300 impact of a land use change on soil biochemical parameters it is necessary to sample far  
301 from soil treatments (Picci and Nannipieri, 2002). This fact, along with the fact that for  
302 measuring such parameters it is recommended to sample far from extreme events (i.e.,  
303 high rainfall), supported our choice (Conant et al., 2000; Picci e Nannipieri, 2002). Daily  
304 mean temperatures and rainfalls during the sampling period (from May to August 2012)  
305 were recorded. Measurements were made between 8 a.m. and 12 a.m., because it is  
306 showed that mid-day values of  $CO_2$  flux is representative of daily average (Davidson et  
307 al., 1998; Luo and Zhou, 2006). Sampling started ten days after collars insertion.

308

#### 309 *2.3.4. Modeling soil respiration*

310 The cumulated total and heterotrophic soil respiration (from 14 May to 13  
311 August 2012) were calculated using the exponential relationship between soil CO<sub>2</sub> flux  
312 and air temperature (van't Hoff, 1884, Arrhenius 1898). The Van't Hoff empirical  
313 exponential equation (Q<sub>10</sub>) (van't Hoff, 1884), a simplified version of the Rothamsted  
314 Carbon Model (RothC; with temperature as independent variable (Coleman and  
315 Jenkinson, 1999) and the Lloyd and Taylor (LT) (Lloyd and Taylor, 1994) models were  
316 used in order to assess the sensitivity of soil respiration to air temperature. The three  
317 models were fitted on measured soil CO<sub>2</sub> fluxes of total and heterotrophic respiration  
318 for both land uses and correlated with air temperature.

319 The performance of the three models of CO<sub>2</sub> flux response to air temperature was  
320 evaluated using different model selection criteria: the Akaike Information Criterion  
321 (AIC); the Root Mean Squared Error (RMSE) and the Adjusted R-square value  
322 (R<sub>sd.ad</sub>). The best statistical fit was chosen to calculate the cumulate flux.

323

#### 324 *2.4. Data and sequence analysis*

325 An updated AM fungal dataset of 59 NS31/AM1 public sequences (ca. 550 bp) was  
326 created, including the majority of the AM fungal species listed in the phylotaxonomic  
327 classification of Schüßler and Walker (2010) and Krüger et al. (2012) (this alignment  
328 has been made accessible in an open-access database  
329 <https://sites.google.com/site/restomedpeatland/microbiology>). The AM fungal dataset  
330 was utilized for the further alignment of the newly generated sequences using Bioedit  
331 (Hall, 1999), after having previously checked the quality of their electropherograms by  
332 Vector NTI Advance 10 (Invitrogen, USA) and their affiliation to the Glomeromycota  
333 phylum by Basic Local Alignment Search Tool (BLAST) search (Altschul et al., 1997).

334 Indeed, a total of 160 newly generated partial SSU Glomeromycota sequences was  
335 aligned with the sequences of the dataset and trimmed to the same length (ca. 490 bp).  
336 Such an alignment, optimized to a total of 195 sequences (34 from the dataset, 160  
337 newly generated and the *Corallochytrium limacisporum* sequence L42528 as outgroup),  
338 was finally manually refined. Phylogenetic trees were inferred by the neighbor-joining  
339 (NJ) analysis using MEGA version 5.1 (Tamura et al., 2011;  
340 <http://www.megasoftware.net>) and the Kimura 2-parameter model (Kimura 1980).  
341 Branch support values correspond to 1000 bootstrap replicates. The phylograms were  
342 drawn by MEGA 5.1 and edited by Adobe Illustrator CS6.

343 The phylogram was utilized to assign the newly generated AM fungal sequences to  
344 molecular operational taxonomic units (MOTUs) on the basis of a bootstrap value of 75.  
345 AM fungal MOTU richness and Shannon index ( $H'$ ) were calculated using Primer v6  
346 (Clarke and Gorley, 2006; <http://www.primer-e.com>). The adequacy of the AM fungal  
347 community sampling was verified using individual-based rarefaction curves (Gotelli &  
348 Colwell, 2001) with clones/sequences considered as units of replication, and was  
349 calculated in EstimateS version 8.2 (Colwell et al., 1997;  
350 <http://viceroy.eeb.uconn.edu/EstimateS>) using the Coleman rarefaction curves  
351 (Coleman, 1981).

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

356 Soil physico-chemical parameters, root DW, AM fungal root colonization, soil and  
357 root AM fungal relative abundances of MOTUs, AM fungal MOTU richness,  $H'$  and  
358 cumulated soil CO<sub>2</sub> fluxes were analyzed by the independent sample  $t$ -test with the land

359 use as factor. In addition, to detect differences among the sampling dates within each  
360 land use, soil CO<sub>2</sub> fluxes were analyzed by the one-way analysis of variance (ANOVA)  
361 with sampling date as factor. All data were log- or arcsin-transformed when necessary  
362 to fulfill the assumption for the *t*-test and the ANOVAs. Post-hoc Tukey's-b significant  
363 difference tests were used for the comparisons among sampling dates. When the  
364 assumptions for the parametric analysis were not fulfilled (soil CO<sub>2</sub> fluxes and the  
365 coefficients of variation of the three components of soil CO<sub>2</sub> fluxes), even after the  
366 appropriate transformation, data were analyzed using the Mann-Whitney nonparametric  
367 test or the Kruskal-Wallis H non-parametric test, followed by the Mann-Whitney test.  
368 All these analyses were performed in SPSS version 21.0 (SPSS Inc., Chicago, IL,  
369 USA).

370 Constrained ordination analyses (Redundancy analysis, RDA) (van den Wollenberg,  
371 1977) were used to investigate the influence of the different land use types (used as  
372 explanatory variables) on the physico-chemical, root and CO<sub>2</sub> parameters and on the  
373 AM fungal relative abundances of MOTUs (used as response variables). We utilized the  
374 RDA linear method (Lepš and Šmilauer, 2003) since the response variables were in  
375 different measurement units and the length of the gradient of the detrended  
376 correspondence analysis was lower than four. All data were log-transformed, centered  
377 and standardized by the response variables. Monte Carlo permutation tests were  
378 performed using 499 random permutations (unrestricted permutation) in order to  
379 determine the statistical significance of the relations between the land use types and the  
380 response variables. RDA analyses were done by Canoco for Windows v. 4.5 (terBraak  
381 and Šmilauer, 2002). The biplots were drawn by CanoDraw for Windows.

382

### 383 **3. Results**

384 *3.1. Experiment 1: evaluation of the impact of land use on soil physico-chemical*  
385 *parameters, roots and AMF*

386

387 *3.1.1. Soil physico-chemical parameters, root dry weight and AM fungal root*  
388 *colonization*

389 Unexpectedly, 15 years of continuous maize (ConvMaize) did not significantly  
390 modified soil quality, as measured by its main physico-chemical parameters, in  
391 comparison with the land abandonment (Aband) except for the soil  $\text{NH}_4^+$  concentration  
392 that was around three-fold higher in the ConvMaize than in the Aband (Table 2).  
393 However, it is noteworthy to highlight a decline trend of the vital component of a  
394 healthy soil. Actually, the ConvMaize land use reduced soil organic matter (SOM) by  
395 9% in comparison with the Aband, as well as for other chemical parameters, such as the  
396  $\text{P}_{\text{tot}}$  and  $\text{P}_{\text{avail}}$  that were reduced by 8% and 5%, respectively.

397 As regard to roots, dry weight values (DW) were not significantly different between  
398 land uses, whereas AM fungal root colonization percentages were significantly lower (-  
399 30%) in the ConvMaize than in the Aband Table 3.

400

401 *3.1.2. AM fungal diversity*

402 The PCR primer pair NS31/AM1, which targets the 3' end of the SSU rRNA gene ( $\approx$   
403 550 bp), was used to amplify and screen the clone libraries obtained from the 12 crude  
404 DNA extracts of field root and soil samples. A total of 366 clones were screened and  
405 270 showed the expected AM fungal band length. In detail, 138 positive clones were  
406 obtained from the ConvMaize (65 and 73 from root (R) and soil samples (S),  
407 respectively) and 132 from the Aband (63 e 69 from R and S, respectively). After

408 sequencing and BLAST checking, about 40% of the sequences were excluded due to  
409 sequencing errors or PCR primer unspecificity.

410 The obtained sequences were grouped into 11 different AM fungal molecular  
411 operational taxonomic units (MOTUs) (a total of eight and seven in the ConvMaize and  
412 the Aband and six and eight in roots and soil, respectively) which were phylogenetically  
413 affiliated to *Funneliformis mosseae* (Fun1\_AMASS), 5 different *Glomus* spp. (from  
414 Glo1\_AMASS to Glo5\_AMASS), *Rhizophagus manihotis* (Rhizo1\_AMASS),  
415 *Rhizophagus irregularis* (Rhizo2\_AMASS), *Sclerocystis sinuosa* (Sclero1\_AMASS),  
416 *Scutellospora dipurpurescens* (Scut1\_AMASS) and an uncultured Glomeromycota  
417 (Uncult1\_AMASS) (Fig. 1; Fig. S3). Three out of 11 MOTUs, although three  
418 doubletons (Glo5\_AMASS, Rhizo1\_AMASS and Scut1\_AMASS), were considered for  
419 further analyses.

420 The rarefaction curves showing the relation between the number of sampled  
421 sequences and the number of observed AM fungal MOTUs retrieved from roots and soil  
422 of the ConvMaize and the Aband land uses (Fig. S4) demonstrated that the sampling  
423 effort was sufficient as the accumulation curves reached the asymptote.

424 As shown in the pie charts of Fig. 1, four AM fungal MOTU were exclusively  
425 retrieved in the soil of the ConvMaize (Glo2\_AMASS, Glo5\_AMASS,  
426 Rhizo1\_AMASS and Sclero1\_AMASS), while three were only found in the Aband:  
427 Fun1\_AMASS in the soil and the others (Scut1\_AMASS and Uncult1\_AMASS) within  
428 the roots. By contrast, Rhizo2\_AMASS, affiliated to *R. irregularis*, showed a  
429 ubiquitous behavior. In fact, it was retrieved in both land uses and matrixes. Along with  
430 Rhizo2\_AMASS, Glo1\_AMASS and Glo4\_AMASS occurred into the soil of both land  
431 uses and within the roots of only the Aband, while Glo3\_AMASS was present in both  
432 land uses but just into the roots.

433 The MOTU richness and Shannon biodiversity index ( $H'$ ) were calculated to evaluate  
434 the long-term sustainability of the two different land uses on AM fungal community  
435 diversity, considering in this way, not only the number, but also the relative proportions  
436 of taxa (Table 4). Unexpectedly, we observed a significantly higher AM fungal richness  
437 and diversity into the soil of the ConvMaize than in the Aband. Interestingly, in the  
438 continuous maize, the soil showed higher values of both indexes respect to the roots.  
439 Along with this, considering the relative proportions of the AM fungal MOTU (Fig. 2  
440 and Table S1), Glo2\_AMASS and Sclero1\_AMASS were significantly more abundant  
441 into the soil of the ConvMaize than in the Aband, while Fun1\_AMASS and  
442 Glo1\_AMASS were significantly less abundant. In fact, Glo2\_AMASS and  
443 Sclero1\_AMASS, which were exclusively found in the ConvMaize soil, showed  
444 relative abundances of 16.9% and 40.9%, respectively, whereas Fun1\_AMASS,  
445 exclusively found in the Aband soil, showed a relative abundance of 21.0%. Along with  
446 Fun1\_AMASS, Glo1\_AMASS was three-fold more present in the Aband than in the  
447 ConvMaize.

448 As regard to root, although a differential trend was found and both diversity indexes  
449 were three-fold higher in the Aband than in the ConvMaize, 15 years of land use change  
450 did not significantly affect AM fungal community richness and evenness (Table 4).

451 The t- or Mann-Whitney tests utilized to highlight the AM fungal relative abundance  
452 difference between matrixes within each land use, showed that, for the ConvMaize,  
453 Glo1\_AMASS, Glo2\_AMASS, Glo4\_AMASS and Sclero1\_AMASS were significantly  
454 more abundant in the soil than in the roots, whereas an opposite trend was observed for  
455 Rhizo2\_AMASS (Table S1). Similarly, Rhizo2\_AMASS was more present into the  
456 roots than in the soil also in the Aband land use, while Fun1\_AMASS and  
457 Glo1\_AMASS showed an opposite behavior.

458 RDAs, in line with the univariate tests, showed that land use change significantly  
459 affected AM fungal composition and structure in the soil and that the different matrixes  
460 have different AM fungal assemblages in both land uses. In detail, RDA showed that in  
461 the soil land use explained 83.5% (I and II axes) of the whole variance (Fig. 3a), and  
462 that its effect on the AM fungal communities was significant ( $P = 0.002$ ), while in the  
463 roots no differences were recorded ( $P = 0.410$ ) (data not shown). With regard to soil  
464 AM fungal assemblages, the arrows representing Glo2\_AMASS, Sclero1\_AMASS,  
465 Glo5\_AMASS and Rhizo1\_AMASS point to the ConvMaize, while those representing  
466 Glo1\_AMASS and Fun1\_AMASS point to the Aband, showing their preferential  
467 presence in the corresponding land use (Fig. 3a). Moreover, Glo4\_AMASS and  
468 Rhizo2\_AMASS did not show preferential behaviors. As regard to the ConvMaize and  
469 the Aband, RDAs showed that the different matrixes explained 89.7% and 89.2% (I and  
470 II axes) of the whole variance, respectively (Fig. 3b,c), and that their effect on the AM  
471 fungal communities was significant ( $P = 0.002$ ). As shown by the arrows, in the  
472 ConvMaizeGlo2\_AMASS, Sclero1\_AMASS, Glo1\_AMASS, Glo4\_AMASS,  
473 Glo5\_AMASS and Rhizo1\_AMASS are soil preferential, while Glo3\_AMASS and  
474 Rhizo2\_AMASS are root preferential (Fig. 3b). Along with this, also in the Aband, the  
475 arrows showed that Rhizo2\_AMASS and Glo3\_AMASS are preferential of the roots  
476 and that Glo1\_AMASS of the soil (Fig. 3c). Interestingly, such an RDA biplot  
477 highlighted that Scut1\_AMASS/Uncult1\_AMASS and Fun1\_AMASS, exclusively  
478 present in the Aband, were preferential of the roots and of the soil, respectively.

479

## 480 3.2. Experiment 2: evaluation of the impact of land use on soil CO<sub>2</sub> fluxes

481

### 482 3.2.1. Soil CO<sub>2</sub> flux measurements

483 Both air and soil temperature and soil moisture strongly varied during the monitoring  
484 period (Fig. 4a,b). From May to August, the mean air and soil temperature ranged from  
485 12.0 to 26.6 °C and from 16.1 to 30.2 °C, respectively; soil moisture (% v:v) decreased  
486 in both land uses from 30.8 to 15.8% and from 27.5 to 2.2% for the ConvMaize and the  
487 Aband, respectively (Fig. 4b). Soil temperatures of the ConvMaize and the Aband  
488 showed significant relationships ( $P < 0.001$ ) with air temperature, as shown by the  
489 Pearson correlation ( $r = 84\%$  and  $90\%$ , respectively) (data not shown). Total soil  
490 respiration ( $R_s$ ), heterotrophic respiration ( $R_h$ ) and autotrophic respiration ( $R_a$ ) steadily  
491 increased following the trend of air and soil temperature (Fig. 4c,d,e).  $R_s$  ranged from  
492 9.74 to 63.35  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$  and from 19.97 to 54.95  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$  in the  
493 ConvMaize and the Aband, respectively, while  $R_h$  from 6.79 to 29.12  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$   
494 and from 17.34 to 35.43  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$  in the ConvMaize and the Aband, respectively.  
495 The ConvMaize showed significantly lower  $R_s$  and  $R_h$  values in the first period of the  
496 monitoring (from the middle to the end of May) than the Aband, whereas, later, we  
497 observed an opposite trend of the  $R_s$ . The  $R_a$  ranged from 2.33 to 38.55  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$   
498 and from 2.63 to 27.82  $\text{g CO}_2 \text{ m}^{-2} \text{ day}^{-1}$  in the ConvMaize and the Aband, respectively.  
499 Actually,  $R_s$  and also  $R_a$  in the ConvMaize were significantly higher than in the Aband  
500 in the second part of both June and July (Fig. 4c,e). By contrast,  $R_h$  values were  
501 significantly higher in the Aband than in the ConvMaize in almost all July (Fig. 4d).

502

### 503 *3.2.2. Model selection of soil CO<sub>2</sub> flux response to air temperature*

504 To calculate the cumulated values of the two measured components ( $R_s$  and  $R_h$ ) of  
505 the soil CO<sub>2</sub> respiration we made a selection of the best model among the most used  
506 ones: Q10, LT and simplified RothC. These models were compared on the basis of three  
507 selection criteria, such as AIC, RSME and Rsq.ad (Table 5). In detail, the LT model

508 showed the best goodness of fit for Rs and Rh in the ConvMaize and the Aband.  
509 Actually, the LT model minimized the AIC and the RSME values, while the simplified  
510 RothC model maximized the Rsq.ad values (Table 5). Therefore, the LT model was  
511 selected for modeling the soil CO<sub>2</sub> flux response to air temperature.

512 The plots of the relationships between the measured Rs and Rh and air temperature  
513 obtained utilizing the LT model are shown in Fig. S5. Clear relationships between Rs  
514 and Rh fluxes and air temperature were observed both in the ConvMaize and the Aband.  
515 Actually, in the ConvMaize strong and significant relationships were revealed (Rs  
516 Rsq.ad = 0.7; Rh Rsq.ad = 0.8;  $P < 0.001$ ) (Table 5; Fig. S5a,c), while in the Aband  
517 significant relationships from moderate to weak were observed (Rs Rsq.ad = 0.5; Rh  
518 Rsq.ad = 0.3;  $P < 0.001$ ) (Table 5; Fig. S5b,d, respectively).

519

### 520 *3.2.3. Impact of land use change on the resilience of CO<sub>2</sub> fluxes and on cumulated soil* 521 *CO<sub>2</sub> fluxes*

522 The coefficients of variation of the Rs and Rh components were significantly higher in  
523 the ConvMaize than in the Aband, showing its lower resilience to air temperature  
524 changes in terms of soil CO<sub>2</sub> flux response (Table 6).

525 The curves of the Rs, Rh and Ra cumulated CO<sub>2</sub> fluxes are shown in Fig. 5. In the  
526 ConvMaize Rs and Ra cumulated CO<sub>2</sub> fluxes (4360 g m<sup>-2</sup> period<sup>-1</sup> and 2316 g m<sup>-2</sup>  
527 period<sup>-1</sup>, respectively) were significantly higher than in the Aband (3999 g m<sup>-2</sup> period<sup>-1</sup>  
528 and 1419 g m<sup>-2</sup> period<sup>-1</sup>, respectively) (Fig. 5). In detail, Rs and Ra cumulated soil CO<sub>2</sub>  
529 flux increases, calculated as  $((\text{ConvMaize} - \text{Aband}) / \text{Aband}) \times 100$ , were 9 and 63%,  
530 respectively. By contrast, the Rh component showed an opposite behavior, showing  
531 significantly lower values in the ConvMaize than in the Aband (- 21%). Interestingly, in

532 the ConvMaize, the Rh and Ra soil CO<sub>2</sub> flux partitionings were 47% and 53%,  
533 respectively, whereas in the Aband 65% and 35%, respectively.

534

535 *3.3. Main patterns of soil physico-chemical parameters, CO<sub>2</sub> fluxes and AM fungal*  
536 *diversity as affected by land use*

537 RDA showed that land use explained 69.5% (I and II axes) of the whole variance and  
538 that its effect on soil quality parameters was significant ( $P = 0.002$ ), as shown by the  
539 biplot arrows representing the ConvMaize and the Aband (Fig. 3d). The biplot clearly  
540 suggested that NH<sub>4</sub><sup>+</sup>, Ra, MOTU richness and H' of soil and Rs, showing higher values  
541 in the ConvMaize respect to the Aband, and Rh and AM fungal colonization, showing  
542 lower values in the ConvMaize respect to the Aband, where the most discriminant  
543 variables between the two land uses.

544

#### 545 **4. Discussion**

546 In this work we assessed for the first time the long-term effect of the land use change,  
547 from a conventional continuous maize to uncultivated conditions, on the soil quality of  
548 a drained Mediterranean peatland, belonging to the Massaciucoli wetland area.

549 Multivariate analyses showed that 15 years of conventional continuous maize  
550 (ConvMaize): (i) did not affect the main soil physico-chemical parameters, except for  
551 the NH<sub>4</sub><sup>+</sup> that was higher in the ConvMaize than in the ex-arable cropping land  
552 (Aband); (ii) decreased the AM fungal root colonization and the root AM fungal  
553 taxonomic diversity, whereas increased the MOTU richness and the Shannon diversity  
554 index of the soil; (iii) enhanced soil and root respiration together with a decrease of the  
555 microbial and mesofauna respiration component; (iv) reduced soil resilience to air  
556 temperature changes in terms of soil CO<sub>2</sub> flux response.

557

558 *4.1. Evaluation of the impact of land use on soil physico-chemical parameters, roots*  
559 *and AMF (Experiment 1)*

560

561 *4.1.1. Soil physico-chemical parameters*

562 The application of alternative farming, based on conservative tillage, fertilization  
563 adjusted to soil nutrient traits and crop nutrient needs, agroecological friendly weed and  
564 pesticide management, have been largely shown to lead to sustainability of  
565 agroecosystems (Altieri, 1995; Matson et al., 1997; Tilman et al., 2002; Schjønning et  
566 al., 2004). Such sustainability is determined not only by the positive effects on the  
567 economic aspects, including crop productivity, but also on the environmental quality,  
568 including air, water and soil quality. So far, land use change and intensification of  
569 cropping systems has been largely studied using long-term experiments mainly focusing  
570 on the effects of tillage and fertilization (Pulleman et al., 2000; Liebig et al., 2004;  
571 Nassi o Di Nasso et al., 2011; Pellegrino et al., 2011). Our work confirmed previous  
572 results showing no changes of the physico-chemical aspects of soil quality due to  
573 conventional farming respect to uncultivated conditions (Liebig et al., 2004; Pellegrino  
574 et al., 2011; Bell et al., 2012), even in an peculiar environment such as a Mediterranean  
575 peat soil, characterized by a very high content of SOM and total N and P. By contrast,  
576 some authors revealed negative trends of the main soil physico-chemical parameters due  
577 to the intensification of land use (Pulleman et al., 2000; Celik, 2005; Gajić, 2013) and  
578 cropping system (Shrestha et al., 2006; Birkhofer et al., 2008). These inconsistencies  
579 might be attributed to depth and time factors. Actually, changes of the main chemical  
580 parameters, especially of the SOM, after land use conversions are commonly detected in  
581 the first 7.5 cm and may need more than 15 years in order to be discriminate (Liebig et

582 al., 2004; Shrestha et al., 2006; Gajić, 2013). The unique observed difference on  $\text{NH}_4^+$   
583 might be well explained by its high temporal variability (Kieft et al., 1998; Violante,  
584 2000) and sampling closeness to N fertilization.

585

#### 586 *4.1.2. Root dry weight and AM fungal root colonization*

587 Root dry weight was utilized to estimate the differences in the size of the root system  
588 of maize and natural succession vegetation occurring in the conventional continuous  
589 maize system (ConvMaize) and in the ex-arable cropping land (Aband), respectively.  
590 Our data were in the range of those obtained at 0-30 cm depth of wheat, giant reed and  
591 miscanthus (0.07-0.2, 0.4-1.6 and 2.9-4.3  $\text{mg g}^{-1}$ , respectively; unpublished data) and  
592 for maize and uncultivated natural weed fallow (0.04-1.8  $\text{mg g}^{-1}$ ) (Anderson, 1988;  
593 Jonsson et al. 1988; Kothari et al., 1990; Mekonnen et al. 1997).

594 Maize has a root apparatus, composed by embryonic primary and seminal roots and  
595 postembryonic shoot-borne and lateral roots, showing in the field strong AM fungal  
596 root colonization (Gavito and Varela, 1993; Hochholdinger et al., 2004). Here, although  
597 the well known highly mycotrophic status of the maize, its AM fungal root colonization  
598 was lower than that in the roots of the natural succession vegetation, occurring in the  
599 ex-arable cropping land. Actually, the difference between the ConvMaize and the  
600 Aband AM fungal root colonization rates can not be explained by plant species  
601 composition, as the 18 plant species occurring in the Aband belonged to 16 different  
602 families (Amaranthaceae, Apiaceae, Asteraceae, Cannabaceae, Caryophyllaceae,  
603 Chenopodiaceae, Convolvulaceae, Lythraceae, Malvaceae, Phytolaccaceae, Poaceae,  
604 Polygonaceae, Rubiaceae, Scrophulariaceae, Solanaceae, Typhaceae) mostly showing a  
605 non-mycorrhizal status (Harley and Harley, 1987). Therefore, tillage, fertilization and  
606 mechanical and chemical weeding, carried out in the ConvMaize, can well explain such

607 a difference because they have been largely shown to reduce mycorrhizal infection  
608 potential of soil by “pluoghing up” the mycelium network (Helgason et al., 1998, 2007;  
609 Jansa et al., 2002; Borriello et al., 2012; Verbruggen et al., 2012).

610

#### 611 *4.1.3. AM fungal diversity*

612 Although in the past the importance of AMF in wetlands has not been taken into  
613 account (Keddy et al., 2000), our study on AM fungal diversity of a Mediterranean  
614 drained peatland was boosted by the increasing awareness of their occurrence and  
615 functionality in these key ecosystems (Miller, 2000; Cornwell et al., 2001; Wolfe et al.,  
616 2007).

617 Different molecular markers belonging to the rRNA gene, such as the SSU, the  
618 internal transcribed spacers (ITS1, 5.8S and ITS2) and the large subunit rRNA gene  
619 (LSU), have been utilized to identify the glomeromycotan species and investigate their  
620 phylogenetical diversity (Helgason et al., 1998; van Tuinen et al., 1998; Redecker et al.,  
621 2003; Gollotte et al., 2004; Lee et al., 2008). Although the relatively lower variability  
622 and species resolution power of the AM fungal NS31/AM1 SSU sequences and the  
623 exclusion of basal lineages of Glomeromycota, such as Archeosporaceae and  
624 Paraglomeraceae, this region is still utilized because it provides a larger comparative  
625 sequence dataset than those available for the other single or combined genomic regions  
626 (Opik et al., 2010; Stockinger et al., 2010; Borriello et al., 2012; Krüger et al., 2012;  
627 Pellegrino et al., 2012). Therefore, since we aimed to study the AM fungal diversity in a  
628 Mediterranean peatland for the first time, we selected the NS31/AM1 region. Here, in  
629 addition to the root matrix that gives a picture of the symbiotically active community,  
630 we carried out the direct extraction of the “environmental” DNA from the usually less

631 investigated soil to avoid the host effect selection (Borriello et al., 2012; Davison et al.,  
632 2012).

633 Root and soil MOTU richness and Shannon index fell into similar range of previous  
634 works (Helgason et al., 1998; Daniell et al., 2001; Husband et al., 2002;  
635 Vandenkoornuyse et al., 2002; Hijri et al., 2006; Borriello et al., 2012), but were largely  
636 lower than those reported by others (Bever et al., 1996; Abbott and Robson, 1997; Jansa  
637 et al., 2002; Wolfe et al., 2007; Mummey and Rillig, 2008; Oehl et al., 2010). These  
638 inconsistencies might be due to the pedo-climatic dissimilarities, but also to the  
639 differences between the methodology of detection. However, focusing on maize  
640 molecular diversity, our data were similar to those reported by several works studying  
641 the effect of tillage and fertilization on the diversity not only in roots, but also in soil  
642 under monocropping systems (Daniell et al., 2001; Hijri et al., 2006; Borriello et al.,  
643 2012). Here, root MOTU richness and Shannon index suggested that AM fungal  
644 diversity associated with the maize “monoculture” is not depleted by the intensification  
645 of cultivation as it had been previously described by field and microcosm studies  
646 (Daniell et al., 2001; Johnson et al., 2004; Hijri et al., 2006). As the diversity of a soil  
647 reflects its accumulated sporulation history and not the current symbionts of the crop,  
648 the ConvMaize, although being a continuous cropping system, showed a higher  
649 diversity respect to the Aband because of the mycorrhizal weed composition (Poaceae,  
650 Solanaceae and Phytolaccaceae). Therefore, these weeds may have supported the AM  
651 fungal root development and the resulting spore composition in the soil of the  
652 ConvMaize.

653 The Glomeraceae and the Acaulosporaceae were commonly and largely retrieved in  
654 agricultural soil, while the Gigasporaceae were more frequently observed in woodland  
655 or uncultivated sites (Helgason et al., 1998; Daniell et al., 2001; Jansa et al., 2002;

656 Gollotte et al., 2004; Hijri et al., 2006; Pellegrino et al., 2011). As regard to the wetland  
657 areas, the Glomeraceae were the most abundant together with few members of the  
658 Acaulosporaceae and the Diversisporaceae (Wirsel et al., 2004; Wolfe et al., 2007). Our  
659 data on AM fungal composition and structure were consistent with the previous  
660 observations. Actually, the Glomeraceae was the unique family retrieved in the soil,  
661 while within the roots a member of the Gigasporaceae was also detected. Within the  
662 Glomeraceae, the dominance of the Rhizo2\_AMASS within the roots of the maize  
663 suggests that it possess an ability to survive in agricultural conditions due to its ability  
664 to colonize the roots from mycelium fragments rather than from spores dispersal or  
665 intact mycelium (Biermann and Linderman, 1983) and to readily form anastomoses that  
666 reestablish the hyphal interconnection after its tillage destruction (De La Providencia et  
667 al., 2005). The presence of a MOTU phylogenetically affiliated to the *S. dipurpurescens*  
668 only within the roots of the natural succession vegetation occurring in the Aband can be  
669 well explained by the soil tillage disruption of the extraradical hyphae that are essential  
670 for the propagation of the Gigasporaceae (Jasper et al., 1993). Despite several authors  
671 highlighted the dominance of *F. mosseae* in arable land, grassland and also wetlands,  
672 unexpectedly we retrieved *F. mosseae* only into the soil of the Aband also without being  
673 the most representative. Interestingly, in the soil of the ConvMaize a MOTU  
674 phylogenetically affiliated to *S. sinuosa* was largely found.

675

#### 676 4.2. Evaluation of the impact of land use on soil CO<sub>2</sub> fluxes (Experiment 2)

677

##### 678 4.2.1. Soil CO<sub>2</sub> flux measurements and cumulated values

679 The differentiation of the soil CO<sub>2</sub> flux into its components, autotrophic and  
680 heterotrophic, has been recently shown to be an important method for evaluating and

681 interpreting soil CO<sub>2</sub> sources (Baggs, 2006; Kuzyakov, 2006). Here, the soil CO<sub>2</sub>  
682 sources were successfully monitored during the maize growing season using the root  
683 exclusion method. In addition, in order to fill respiration gaps and to assess the total  
684 respiration over the monitored period, the air temperature was used as the  
685 environmental variable for CO<sub>2</sub> modelling. In fact, although some authors have modeled  
686 soil respiration by using several environmental parameters such as soil and air  
687 temperature, soil water content and water table depth (Almagro et al., 2009; Correia et  
688 al., 2012; Rowson et al., 2013), most authors found a highly significant relationship  
689 between soil or air temperature and soil respiration (Luo and Zhou, 2006; Richardson et  
690 al., 2006; Subke et al., 2006). In this regard, we selected, on the basis of three different  
691 criteria, the LT model respect to the Q10 and the simplified RothC, in agreement also  
692 with the suggestions of Davidson et al. (2006) and Luo and Zhou (2006).

693 Here, the cumulated values of the total soil respiration (Rs), extrapolated by using the  
694 LT model, were utilized to obtain the means of the daily Rs values. The calculated daily  
695 Rs fluxes of the ConvMaize and the Aband (47.39 and 43.47 g CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>,  
696 respectively) are higher than those reported by several authors (from 0.77 to 26.6 g CO<sub>2</sub>  
697 m<sup>-2</sup> day<sup>-1</sup>) for fens and peatlands in boreal and temperate areas (Moore, 1986; Silvola et  
698 al., 1996; Kasimir-Klemedtsson et al., 1997; Smith et al., 2007; Danivčič et al., 2010;  
699 Berglund et al., 2011; Heinemeyer et al., 2011; Carter et al., 2012; Schrier-Uijl et al.,  
700 2012; Wunderlich et al., 2012). These inconsistencies might be explained by the fact  
701 that we monitored an unexplored habitat such as a Mediterranean peatland, which is  
702 characterized, respect to the boreal and temperate peatlands, by high mineralization rate  
703 and warmer temperatures in the spring-summer period. In addition to such factors, the  
704 not limiting soil water content assessed in our peatland could have determined larger  
705 fluxes respect to those commonly registered in the Mediterranean areas (from 3.19 to

706 25.09 g CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>) (Wang et al., 2000; Tedeschi et al., 2006; Almagro et al., 2009;  
707 Mancinelli et al., 2010; Carter et al., 2012; Correia et al., 2012; Lai et al., 2012).

708 As suggested by Subke et al. (2006) we compared our results on soil CO<sub>2</sub> partitioning  
709 using the Rh/Rs ratio instead of the Ra/Rh ratio because the Rh flux was directly  
710 recorded, whereas the Ra was calculated by difference. The Rh/Rs ratio ranged from  
711 0.47 to 0.65 in the ConvMaize and the Aband, respectively. These ratios were similar to  
712 those reported in Mediterranean croplands, where the mean ratio was around 0.50  
713 (Wang et al., 2000; Subke et al., 2006; Lai et al., 2012). By contrast, our ratios were  
714 lower than those reported for boreal and temperate peatlands, ranging from 0.72 to 0.97  
715 (Silvola et al., 1996; Wunderlich et al., 2012). Consistently with Subke et al. (2006) in  
716 the Aband, the Rh cumulated values were higher than the Ra values (+81%) and similar  
717 to the pristine peatlands, whereas in the ConvMaize we detected an opposite trend (-  
718 12%) similar to the croplands.

719

#### 720 4.2.2. Resilience of the CO<sub>2</sub> fluxes

721 In agreement with our data, comparing the fluctuation of the CO<sub>2</sub> flux in the Aband  
722 respect to the ConvMaize, Jackson et al. (2003) reported a lower resilience in the tilled  
723 cropping systems than in the no-tilled ones. This behavior might be explained by the  
724 higher porosity, the initial lower bulk density and the lower pore connectivity of the  
725 long-term tilled soils compared to the no-till ones that result in slightly warmer  
726 temperatures and in a consequent larger microbial metabolic activity (Silgran and  
727 Shepherd, 1999). Furthermore, Laliberté et al. (2010) linked the reduction of the  
728 resilience following a land-use intensification gradient to plant diversity and  
729 functionality. Along with this, our monocropping maize system showed lower plant  
730 diversity in comparison with the ex-arable cropping land.

731

## 732 **Conclusions**

733 Our study indicates that 15 years of conventional agriculture in a Mediterranean  
734 peatland alter soil quality mainly by decreasing root AM fungal taxonomic diversity,  
735 enhancing total soil CO<sub>2</sub> respiration and reducing resilience of the total and  
736 heterotrophic soil CO<sub>2</sub> fluxes. Although we did not attempt to measure the relationship  
737 between AM fungal diversity and composition and host functionality, we can argue that  
738 the conventional continuous maize might determine a loss of functionality and  
739 reliability linked to AMF in such a vulnerable ecosystem. In addition, the drainage and  
740 cultivation of these peatsoils clearly transform them in a large source of CO<sub>2</sub> due to the  
741 higher degradation rate of the SOM determined by an enhanced microbial activity.  
742 Actually, drainage and soil tillage modify the soil physico-chemical properties  
743 increasing aeration and porosity, resulting in an oxidizing environment and warmer  
744 temperatures that are factors boosting the microbial activity. This is a first direct  
745 confirmation that the biochemical oxidation of degraded peats is a major process  
746 determining the observed subsidence rate (3 to 4 cm yr<sup>-1</sup>). These main findings can be  
747 used to protect and preserve the Mediterranean peatlands and to identify sustainable  
748 solutions for their management.

749

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751

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759

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1150

## 1151 Captions

1152

1153 **Figure 1.** Neighbor-Joining (NJ) tree (Saitou and Nei, 1987) of arbuscular mycorrhizal fungal  
1154 sequences derived from roots and soil (shown as triangle and circle, respectively) of a conventional  
1155 continuous maize system (ConvMaize) and an ex-arable cropping land, being uncultivated for 15  
1156 years (Aband) (red/filled and green/open symbols, respectively). In the ConvMaize roots were  
1157 represented by maize, whereas in the Aband by native plant species. The analysis is based on partial  
1158 nuclear small subunit ribosomal RNA gene sequences (SSU  $\approx$  550 bp; NS31/AM1 fragment), and  
1159 the tree is rooted with a reference sequence of *Corallochytrium limacisporum* (L42528). Clades of  
1160 sequences were affiliated to *Funneliformis mosseae* (8), *Rhizophagus manihotis* (4), *Rhizophagus*  
1161 *irregularis* (5), *Sclerocystis sinuosa* (3), *Scutellospora dipurpurescens* (10) and to additional taxa,  
1162 such as *Glomus* spp. (1, 2, 6, 7, 9), or uncultured Glomeromycota (11). Pies indicate the proportions  
1163 of sequences into the two land uses (ConvMaize, red; Aband, green) and matrixes (roots, light  
1164 color; soil, dark color). The tree is drawn to scale, with branch lengths in the same units as those of  
1165 the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were  
1166 computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number  
1167 of base substitutions per site. Bootstrapping is based on 1000 replicates (Felsenstein, 1985). The  
1168 analysis involved 195 nucleotide sequences. Evolutionary analyses were conducted in MEGA5  
1169 (Tamura et al., 2011). Sequences obtained in the present study are shown by symbols and their  
1170 accession numbers are shown in Fig. S2.

1171

1172 **Figure 2.** Arbuscular mycorrhizal fungal (AMF) community diversity showed as relative  
1173 abundances of molecular operational taxonomic units (MOTUs) in the soil and within the roots of  
1174 native plant species and maize of a conventional continuous maize system (ConvMaize) and an ex-  
1175 arable cropping land, being uncultivated for 15 years (Aband), respectively. AMF diversity is  
1176 represented by the following clades: *Funneliformis mosseae* (Fun1\_AMASS); *Glomus* spp. (from

1177 Glo1\_AMASS to Glo5\_AMASS); *Rhizophagus manihotis* (Rhizo1\_AMASS); *Rhizophagus*  
1178 *irregularis* (Rhizo2\_AMASS); *Sclerocystis sinuosa* (Sclero1\_AMASS); *Scutellospora*  
1179 *dipurpurescens* (Scut1\_AMASS); uncultured Glomeromycota (Uncult1\_AMASS). Numbers in  
1180 brackets are referred to the clades shown in Fig. 1 and Table S1.

1181

1182 **Figure 3.** Redundancy Analysis (RDA) biplot based on the soil relative abundances of arbuscular  
1183 mycorrhizal fungal (AMF) molecular operational taxonomic units (MOTUs) (*Funneliformis*  
1184 *mosseae*, Fun1\_AMASS; *Glomus* spp., from Glo1\_AMASS to Glo5\_AMASS; *Rhizophagus*  
1185 *manihotis*, Rhizo1\_AMASS; *Rhizophagus irregularis*, Rhizo2\_AMASS; *Sclerocystis sinuosa*,  
1186 Sclero1\_AMASS; *Scutellospora dipurpurescens*, Scut1\_AMASS; uncultured Glomeromycota,  
1187 Uncult1\_AMASS), used as response variable, and land uses [a conventional continuous maize  
1188 system (ConvMaize) and an ex-arable cropping land, being uncultivated for 15 years (Aband)] (a).  
1189 RDA plots of the AM fungal MOTUs, used as response variables, and matrixes (soil and roots),  
1190 used as environmental variables in the ConvMaize (b) and the Aband (c). RDA biplot based on soil  
1191 physico-chemical parameters (pH; electrical conductivity, EC; exchangeable potassium,  $K_{\text{exch}}$ ; total  
1192 nitrogen,  $N_{\text{tot}}$ ; ammonium; nitrates; soil organic matter, SOM; carbon/nitrogen ratio, C/N; total  
1193 phosphorus,  $P_{\text{tot}}$ ; available phosphorus,  $P_{\text{avail}}$ ; organic phosphorus,  $P_{\text{org}}$ ); CO<sub>2</sub> fluxes (total soil  
1194 respiration,  $R_s$ ; heterotrophic respiration,  $R_h$ ; autotrophic respiration,  $R_a$ ); root measurements (total  
1195 root dry weight, total root DW; AMF root colonization, AMF coloniz); AMF diversity (AMF  
1196 MOTU richness of roots and soil, MOTUr and MOTUs, respectively; and Shannon index ( $H'$ ) of  
1197 roots and soil,  $H'$  roots and  $H'$  soil, respectively), used as response variables and the land uses,  
1198 ConvMaize and Aband, used as environmental variables (d). The 1<sup>st</sup> and 2<sup>nd</sup> axes accounted for  
1199 83.5%, 89.7%, 89.2% and 69.5% of the total variance explained by all canonical axes for a, b, c and  
1200 d, respectively. The Monte Carlo permutational tests showed that AM fungal assemblages were  
1201 statistically different between the soil of the ConvMaize and of the Aband and between soil and  
1202 roots of both ConvMaize and Aband ( $P = 0.002$ ) (a, b and c) and that such land use were also

1203 statistically different considering soil physico-chemical, CO<sub>2</sub> fluxes, root measurements and AMF  
1204 diversity ( $P = 0.002$ ) (d).

1205

1206 **Figure 4.** Daily maximum, mean and minimum temperatures (°C) and total rainfall (mm) over the  
1207 monitoring campaign (a). Mean soil temperature (°C) (shown as circle) and moisture (%; v:v)  
1208 (shown as square) of a conventional continuous maize system (filled symbols; ConvMaize) and an  
1209 ex-arable cropping land, being uncultivated for 15 years (open symbols; Aband) (b). Soil  
1210 temperature and moisture were measured outside the six blocks (see Fig. S1) used during the CO<sub>2</sub>  
1211 flux monitoring campaign. Three components of the soil CO<sub>2</sub> fluxes, total soil respiration (Rs;  
1212 shown as triangle (c), heterotrophic respiration (Rh; shown as diamond) (d) were measured in the  
1213 two different land uses ConvMaize vs Aband. The autotrophic respiration (Ra; shown as circle) was  
1214 calculated as difference between Rs and Rh. The monitoring campaign ranges from the 14<sup>th</sup> of May  
1215 to the 13<sup>th</sup> of August 2012 with one or two soil CO<sub>2</sub> flux measurements per week ( $n = 21$ ). Values  
1216 are means  $\pm$  SE of three replicate plots for each treatment (land use). For each sampling date and  
1217 soil CO<sub>2</sub> flux component, statistically significant differences between land uses are shown by  
1218 different letters according to the  $t$ -test or the Mann-Whitney non-parametric test ( $P < 0.05$ ). Open  
1219 and filled symbols represent the Aband and the ConvMaize, respectively.

1220

1221 **Figure 5.** Cumulated of three components of the soil CO<sub>2</sub> fluxes: total soil respiration, R<sub>s</sub> (shown as  
1222 triangle); heterotrophic respiration, R<sub>h</sub> (shown as diamond) and autotrophic respiration, R<sub>a</sub> (shown  
1223 as circle). The measurements ( $n=21$ ) were recorded from May to August 2012 in a conventional  
1224 continuous maize (ConvMaize; filled symbols) vs an ex-arable cropping land, being uncultivated  
1225 for 15 years (Aband; open symbols). Values are means of three replicate plots for each treatment  
1226 (management). Statistically significant differences between managements are shown by different  
1227 letters according to the  $t$ -test ( $P < 0.05$ ).

1228

**Table 1**

Texture (0-30 cm depth) of a conventional continuous maize system (ConvMaize) and of an ex-arable cropping land, being uncultivated for 15 years (Aband). Both sites are located in a Mediterranean peatland of the Regional Park of San Rossore-Migliarino-Massaciuccoli, Pisa, Italy.

Parameters	ConvMaize	Aband
clay (%)	23.4 ± 8.5 <sup>a</sup>	26.5 ± 5.0
silt (%)	24.0 ± 1.8	26.0 ± 3.9
sand (%)	52.6 ± 10.0	47.4 ± 8.6

<sup>a</sup>Values are means ± SE of three plots for each treatment. Values are not statistically different according to the *t*-test.

**Table 2**

Soil physico-chemical parameters (0-30 cm depth) of a conventional continuous maize system (ConvMaize) and of an ex-arable cropping land, being uncultivated for 15 years (Aband).

Physico-chemical parameters	ConvMaize	Aband
pH <sup>a</sup>	4.6 <sup>b</sup>	5.3
EC (mS cm <sup>-1</sup> )	1.8	0.9
K <sub>exch</sub> (mg kg <sup>-1</sup> )	397.0	560.0
N <sub>tot</sub> (g kg <sup>-1</sup> )	13.0	11.8
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	42.3	59.0
NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	148.0 b	56.3 a
SOM (%)	25.7	28.3
C/N	11.5	13.7
P <sub>tot</sub> (mg kg <sup>-1</sup> )	2709.0	2846.7
P <sub>avail</sub> (mg kg <sup>-1</sup> )	70.3	76.3
P <sub>org</sub> (mg kg <sup>-1</sup> )	2153.3	2054.7

<sup>a</sup>EC: electrical conductivity; K<sub>exch</sub>: exchangeable potassium; N<sub>tot</sub>: total nitrogen; NO<sub>3</sub><sup>-</sup>: nitrate; NH<sub>4</sub><sup>+</sup>: ammonium; SOM: soil organic matter; C/N: carbon/nitrogen ratio; P<sub>tot</sub>: total phosphorus; P<sub>avail</sub>: available phosphorus; P<sub>org</sub>: organic phosphorus; <sup>b</sup>Values are means ± SE of three plots for each treatment. Values in the same row followed by different letters are statistically different between management according to the *t*-test ( $P < 0.05$ ).

**Table 3**

Root dry weight and arbuscular mycorrhizal (AM) fungal root colonization of maize and of the natural succession vegetation<sup>a</sup> occurring in a conventional continuous maize system (ConvMaize) and in an ex-arable cropping land (Aband), respectively. The Aband was uncultivated for 15 years.

Parameters	ConvMaize	Aband
Root dry weight (mg g <sup>-1</sup> soil)	0.55 <sup>b</sup>	1.08
AM fungal root colonization (%)	22.2 a	33.2 b

<sup>a</sup>Natural succession vegetation was composed by Amarantaceae (*Amaranthus retroflexus* L.); Apiaceae (*Bifora* sp.); Asteraceae (*Arctium lappa* L., *Bidens* sp., *Xanthium* sp., *Artemisia* sp.); Cannabiaceae (*Humulus lupulus* L.); Cariophyllaceae (*Silene alba* L.); Chenopodiaceae (*Atriplex* sp.); Convolvulaceae (*Calistegia* sp.); Lythraceae (*Lythrum salicaria* L.); Malvaceae (*Abutilon theophrasti* L., ); Phytolaccaceae (*Phytolacca americana* L.); Poaceae (*Phragmites australis* L., *Echinochloa crus-galli* L.); Polygonaceae (*Rumex crispus* L.); Rubiaceae (*Galium* sp.); Scrophulariaceae (*Linaria* sp.); Solanaceae (*Datura stramonium* L.); Thyphaceae (*Thypha latifolia* L.); <sup>b</sup> Values are means of three plots for each treatment. Values in the same row followed by different letters are statistically different between managements according to the *t*-test ( $P < 0.05$ ).

**Table 4.** Arbuscular mycorrhizal (AM) fungal molecular operational taxonomic unit (MOTU) richness and Shannon ( $H'$ ) index within the maize roots and the soil of a conventional continuous maize system (ConvMaize) and within the native plant species roots and the soil of an ex-arable cropping land, being uncultivated for 15 years (Aband).

Parameter	Roots		Soil	
	ConvMaize	Aband	ConvMaize	Aband
MOTU richness	1.7 <sup>a</sup> A	4.3	6.0 b B	3.3 a
Shannon index ( $H'$ )	0.4 A	1.1	1.5 b B	1.0 a

<sup>a</sup>Values in the same row followed by different small letters are statistically different between land uses, according to the  $t$ -test ( $P < 0.05$ ).

**Table 5**

Comparison of three models of CO<sub>2</sub> 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 (Rsd.ad). The measurements (n = 21) were recorded from May to August 2012 in a conventional continuous maize system (ConvMaize) and in an ex-arable cropping land, being uncultivated for 15 years (Aband).

Criteria	Q10 <sup>a</sup>		LT		RothC	
	ConvMaize	Aband	ConvMaize	Aband	ConvMaize	Aband
Rs						
AIC	472.9 <sup>b</sup>	317.9	469.9	317.2	531.0	365.3
RSME	9.8	9.1	9.6	9.0	15.9	16.2
Rsq.ad	0.6	0.5	0.7	0.5	0.8	0.7
Rh						
AIC	331.7	296.6	326.4	296.6	384.8	334.5
RSME	3.2	7.1	3.1	7.1	5.0	11.3
Rsq.ad	0.8	0.3	0.8	0.3	0.8	0.7

<sup>a</sup> Q10: Van't Hoff's Q<sub>10</sub> model; LT: Lloyd and Taylor model; RothC: simplified Rothamsted Carbon Model; <sup>b</sup> Fit model values.

**Table 6**

Coefficient of variation of the total soil respiration (Rs) and of the heterotrophic (Rh) and autotrophic respiration (Ra) of two land uses [a conventional continuous maize system (ConvMaize) vs an ex-arable cropping land, being uncultivated for 15 years (Aband)] over the monitoring campaign.

Parameter	Coefficient of variation	
	ConvMaize	Aband
CV_Rs (%)	32.0 <sup>a</sup> b	23.3 a
CV_Rh (%)	28.9 b	19.1 a
CV_Ra (%)	36.7	39.3

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





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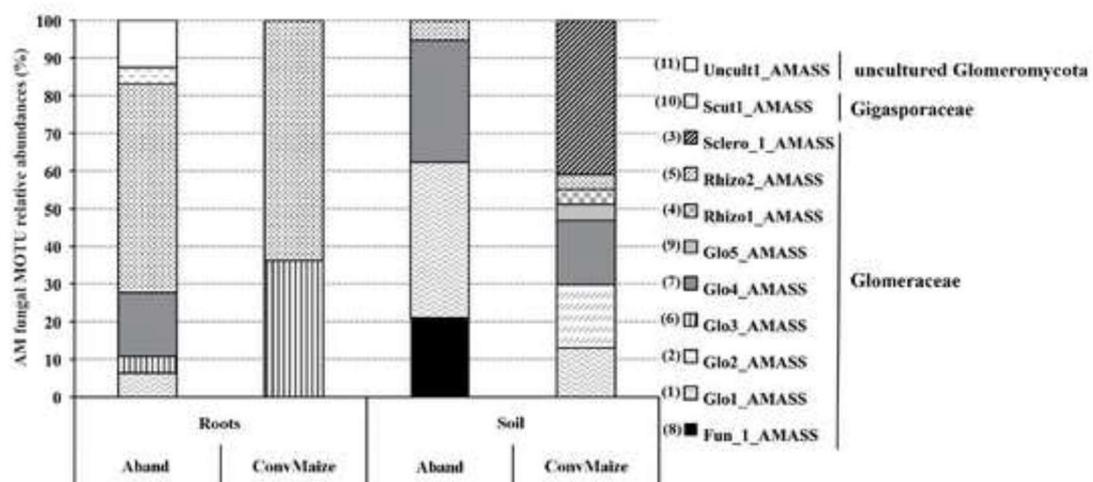


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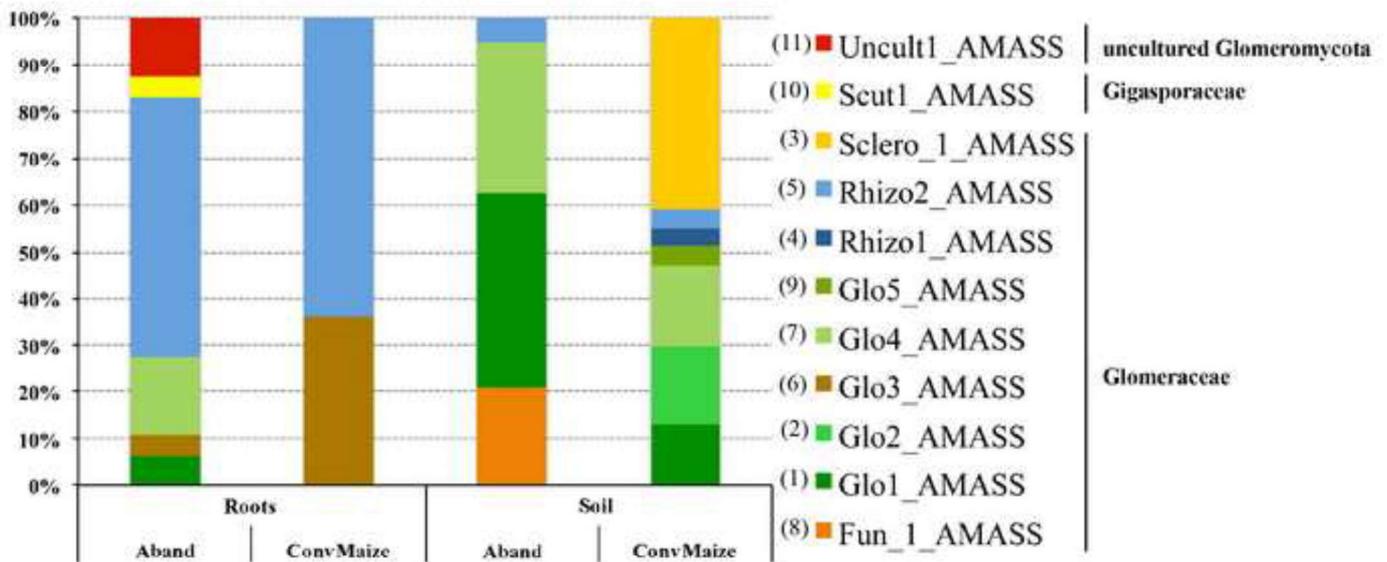




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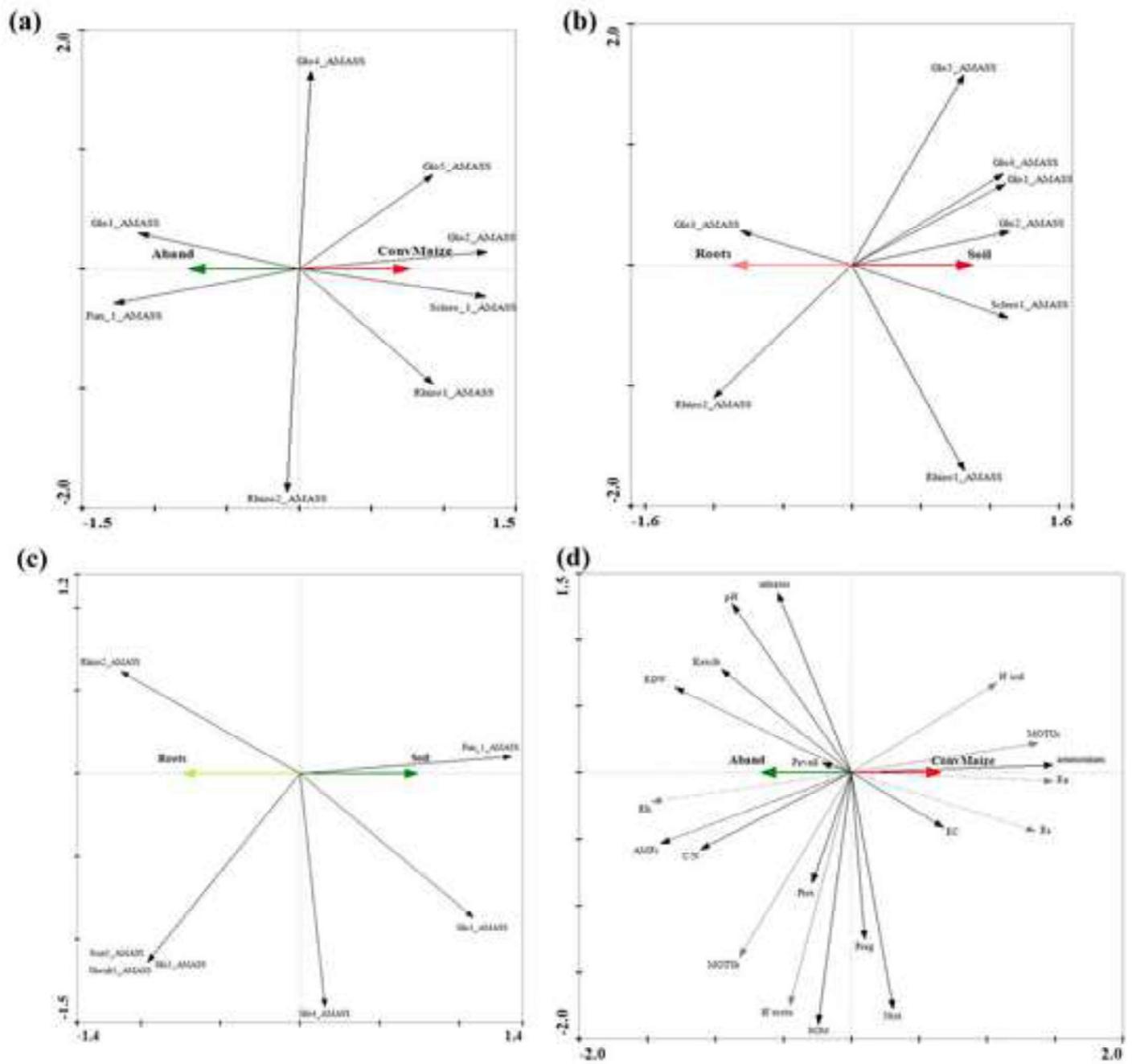


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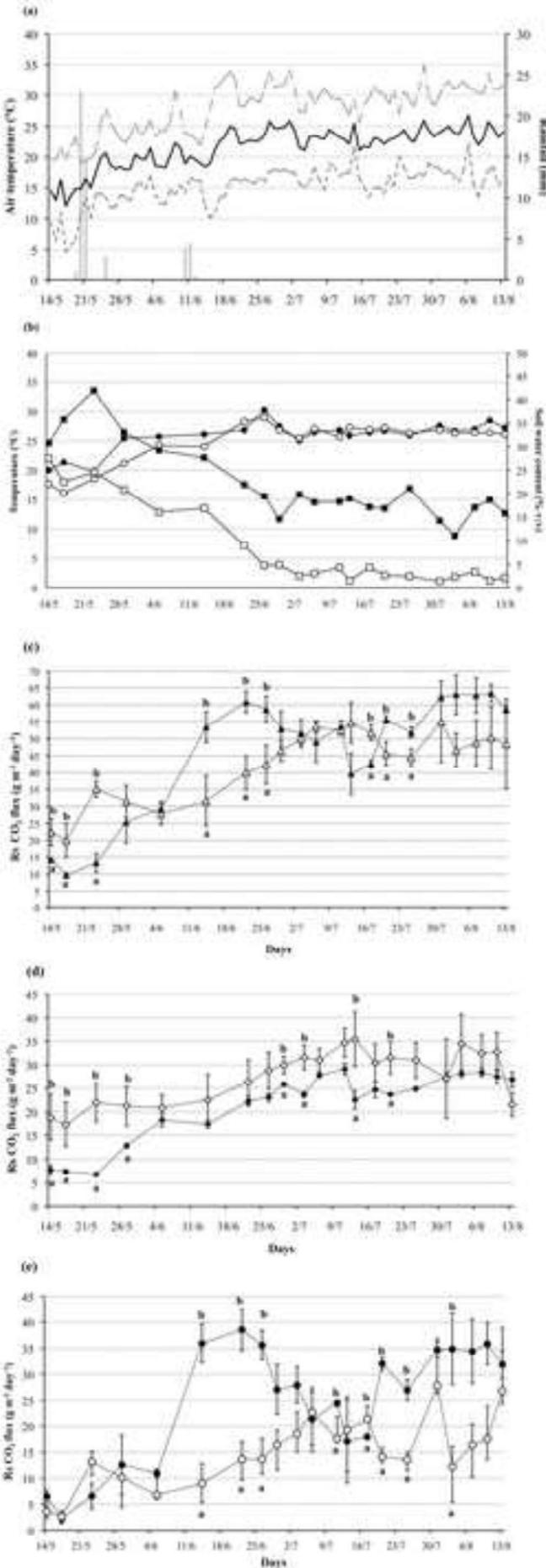


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